

09/719737

Practitioner's Docket No. 701826-051150

CHAPTER II

**TRANSMITTAL LETTER
TO THE UNITED STATES ELECTED OFFICE (EO/US)
(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)**

| | | |
|----------------------------------|---------------------------|--------------------------------------|
| PCT/CA99/00572 | 17 June 1999 (17.06.99) | 17 June 1998 (17.06.98) |
| International Application Number | International Filing Date | International Earliest Priority Date |

TITLE OF INVENTION: ANTISENSE OLIGONUCLEOTIDES FOR TREATING OR PREVENTING
ATOPIC DISEASES AND NEOPLASTIC CELL PROLIFERATION

APPLICANT(S): RENZI, Paolo

CERTIFICATE OF MAILING

I hereby certify that this correspondence, on the date shown below, is being deposited with the United States Postal Service with sufficient postage as Express Mail Label No. EL565095346US in an envelope addressed to Box PCT Assistant Commissioner of Patents, Washington, D.C. 20231, Attention: EO/US.

Date: 15 December 2000

Nicole M. Gignac
Nicole M. Gignac

**Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US**

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:
 - a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. section 371(f)).
 - b. ☒ The U.S. National Fee (35 U.S.C. section 371(c)(1)) and other fees (37 C.F.R. section 1.492) as indicated below:

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2. Fees

| CLAIMS FEE | (1) FOR | (2) NUMBER FILED | (3) NUMBER EXTRA | (4) RATE | (5) CALCULATIONS |
|--------------|--|------------------|------------------|--------------|------------------|
| [X]* | TOTAL CLAIMS | 36 - 20 = | 16 | x \$ 18.00 = | \$288.00 |
| | INDEPENDENT CLAIMS | 10 - 3 = | 7 | x \$ 80.00 = | \$560.00 |
| | MULTIPLE DEPENDENT CLAIM(S) (if applicable) + \$260.00 | | | | |
| BASIC FEE** | <input type="checkbox"/> U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in section 1.482 has been paid on the international application to the U.S. PTO: <input type="checkbox"/> and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(2) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. section 1.492(a)(4)) \$96.00 <input type="checkbox"/> and the above requirements are not met (37 C.F.R. section 1.492(a)(1)) \$670.00 <input checked="" type="checkbox"/> U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in section 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in section 1.445(a)(2) to the U.S. PTO: <input type="checkbox"/> has been paid (37 C.F.R. 1.492(a)(2)) \$760.00 <input type="checkbox"/> has not been paid (37 C.F.R. 1.492(a)(3)) \$970.00 <input checked="" type="checkbox"/> where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. section 1.492(a)(5)) \$860.00 | | | | \$860.00 |
| | Total of above Calculations | | | | = \$1,708.00 |
| SMALL ENTITY | Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed. (note 37 C.F.R. sections 1.9, 1.27, 1.28) Applicant is a Small Entity. | | | | - \$854.00 |
| | Subtotal | | | | \$854.00 |
| | Total National Fee | | | | \$854.00 |
| | Fee for recording the enclosed assignment document \$40.00 (37 C.F.R. 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET". | | | | |
| TOTAL | Total Fees enclosed | | | | \$854.00 |

*See attached Preliminary Amendment Reducing the Number of Claims.

- i. ☒ A check in the amount of \$854.00 to cover the above fees is enclosed.
ii. ☐ Please charge Account No. _____ in the amount of \$ _____.
A duplicate copy of this sheet is enclosed.
3. ☒ A copy of the International application as filed (35 U.S.C. section 371(c)(2)):
- a. ☐ is transmitted herewith.
b. ☐ is not required, as the application was filed with the United States Receiving Office.
c. ☒ has been transmitted
i. ☒ by the International Bureau.
Date of mailing of the application (from form PCT/IB/308): 23 December 1999.
ii. ☐ by applicant on _____.
Date
4. ☒ A translation of the International application into the English language (35 U.S.C. section 371(c)(2)):
- a. ☐ is transmitted herewith.
b. ☒ is not required as the application was filed in English.
c. ☐ was previously transmitted by applicant on _____.
Date
d. ☐ will follow.
5. ☐ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. section 371(c)(3)):
- a. ☐ are transmitted herewith.
b. ☐ have been transmitted
i. ☐ by the International Bureau.
Date of mailing of the amendment (from form PCT/IB/308): _____.
ii. ☐ by applicant on _____.
Date
c. ☐ have not been transmitted as
i. ☐ applicant chose not to make amendments under PCT Article 19.
Date of mailing of Search Report (from form PCT/ISA/210): _____.
ii. ☐ the time limit for the submission of amendments has not yet expired.
The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

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6. ☐ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. section 371(c)(3)):
- a. ☐ is transmitted herewith.
 - b. ☐ is not required as the amendments were made in the English language.
 - c. ☐ has not been transmitted for reasons indicated at point 5(c) above.
7. ☒ A copy of the international examination report (PCT/IPEA/409)
- ☒ is transmitted herewith.
 - ☐ is not required as the application was filed with the United States Receiving Office.
8. ☒ Annex(es) to the international preliminary examination report
- a. ☒ is/are transmitted herewith.
 - b. ☐ is/are not required as the application was filed with the United States Receiving Office.
9. ☒ A translation of the annexes to the international preliminary examination report
- a. ☐ is transmitted herewith.
 - b. ☒ is not required as the annexes are in the English language.
10. ☒ An oath or declaration of the inventor (35 U.S.C. section 371(c)(4)) complying with 35 U.S.C. 115
- a. ☐ was previously submitted by applicant on _____.
Date
 - b. ☐ is submitted herewith, and such oath or declaration
 - i. ☐ is attached to the application.
 - ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. section 1.70.
 - c. ☒ will follow.

Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):
- a. ☒ is transmitted herewith.
 - b. ☐ has been transmitted by the International Bureau.
Date of mailing (from form PCT/IB/308): _____.
 - c. ☐ is not required, as the application was searched by the United States International Searching Authority.
 - d. ☐ will be transmitted promptly upon request.
 - e. ☐ has been submitted by applicant on _____.

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Date

12. ☐ An Information Disclosure Statement under 37 C.F.R. sections 1.97 and 1.98:
- a. ☐ is transmitted herewith.
Also transmitted herewith is/are:
☐ Form PTO-1449 (PTO/SB/08A and 08B).
☐ Copies of citations listed.
- b. ☐ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. sections 371(c).
- c. ☐ was previously submitted by applicant on _____.
Date

13. ☐ An assignment document is transmitted herewith for recording.

A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING
NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also
attached.

14. ☒ Additional documents:
- a. ☒ Copy of request (PCT/RO/101)
- b. ☒ International Publication No. WO 99/66037
- i. ☐ Specification, claims and drawing
- ii. ☐ Front page only
- c. ☒ Preliminary amendment (37 C.F.R. section 1.121)
- d. ☒ Other

Response to Written Opinion

15. ☒ The above checked items are being transmitted
- a. ☒ before 30 months from any claimed priority date.
- b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on
_____, namely:

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AUTHORIZATION TO CHARGE ADDITIONAL FEES

[X] The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 50-0850.

- [X] 37 C.F.R. 1.492(a)(1), (2), (3), and (4) (filing fees)
- [X] 37 C.F.R. 1.492(b), (c) and (d) (presentation of extra claims)
- [X] 37 C.F.R. 1.17 (application processing fees)
- [] 37 C.F.R. 1.17(a)(1)-(5)(extension fees pursuant to section 1.136(a).
- [] 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))
- [] 37 C.F.R. section 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

Date: 15 December 2000

 Reg. No. 3423.5

David S. Resnick
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101 Federal Street
Boston, MA 02110
Tel: (617) 345-1000, X6057
Fax: (617) 345-1300

09/719737-062901

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Paolo RenziFor: **ANTISENSE OLIGONUCLEOTIDES FOR TREATING OR
PREVENTING ATOPIC DISEASES AND NEOPLASTIC CELL
PROLIFERATION**Filed: **June 17, 1999**Serial No.: **09/719,737**Examiner: **Vonda M. Wallace**

The Commissioner of Patents
and Trademarks
Washington, D.C. 20231
U.S.A.

STATEMENT UNDER 1.821(f)

Sir:

In connection with this Application a Computer Readable Copy of the Information required under 1.821 is being submitted concurrently together with the specification pages preceding the claims.

As required under 1.821(f), the undersigned hereby states that the content of the paper copy which comprises the General Information and the Sequence Listings and the Computer Readable Copy are the same and does not contain new matter.

This statement is made by a person registered to practice before the U.S. Patent and Trademark Office and as such, a verified statement is not required and is not being submitted.

Respectfully,

By: 

Christian Cawthorn
Reg. No. 47,352
Tel.: (514) 847-4256

Date: June 27, 2001

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Paolo Renzi
SERIAL NO.: 09/719,737 GROUP: -
Filed: June 17, 1999 EXAMINER: Vonda M. Wallace
FOR: ANTISENSE OLIGONUCLEOTIDES FOR TREATING
OR PREVENTING ATOPIC DISEASES AND NEOPLASTIC
CELL PROLIFERATION

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, DC 20231

Sir:

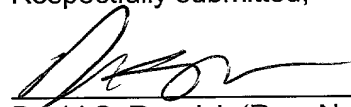
RESPONSE TO SEQUENCE LISTING REQUIREMENT

In response to the Notice to Comply with the Sequence Listing Requirements mailed April 30, 2001, a copy of which is attached hereto, Applicant encloses herewith a paper copy and a computer readable form of the "Sequence Listing" and a statement that the content of the paper and computer readable copies are the same and does not contain new matter.

The Commissioner is hereby authorized to charge any additional fees associated with this communication to Deposit Account No. 50-0820.

Respectfully submitted,

Date: 6/29/01



David S. Resnick (Reg. No. 34,235)
Nixon Peabody LLP
101 Federal Street
Boston, Massachusetts 02110-1832
(617) 345-6057

Encl.: - Pages 1 to 6 of the Sequence Listing;
- Computer-readable form of the Sequence Listing; and
- Statement.

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DOCKET NO. 701826-051105

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: RENZI, Paolo

Application No.: Herewith
(U.S. National Phase of PCT/CA99/00572)

Group No.: Not yet assigned

Filed: Herewith
(International Filing Date: 17 June 1999)

Examiner: Not yet assigned

For: ANTISENSE OLIGONUCLEOTIDES FOR TREATING OR PREVENTING ATOPIC
DISEASES AND NEOPLASTIC CELL PROLIFERATION

CERTIFICATE OF MAILING

I hereby certify that this correspondence, on the date shown below, is being deposited with the United States Postal Service with sufficient postage as Express Mail Label No. EL565095346US in an envelope addressed to Box PCT Assistant Commissioner of Patents, Washington, D.C. 20231, Attention: EO/US.

Date: 15 December 2000


Nicole M. Gignac

PRELIMINARY AMENDMENT

This Preliminary Amendment is being filed in the U.S. Patent and Trademark Office.

Please amend the following application as follows:

IN THE CLAIMS:

Please cancel the current claims and add the following new claims:

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1. An antisense oligonucleotide for treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, said oligonucleotide being directed against a nucleic acid sequence coding for a common subunit of the IL-3, IL-5 and GM-CSF receptors.
2. The oligonucleotide of claim 1, wherein the nucleic acid sequence coding for the receptor is a nucleic acid coding for the common beta sub-unit of the IL-3, IL-5 and GM-CSF receptors.
- 3 The oligonucleotide of claim 1, wherein said oligonucleotide has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, , SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 and SEQ ID NO:23.
4. A pharmaceutical composition for treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, said composition comprising at least one antisense oligonucleotide as defined in claim 1, 2 or 3, in association with a pharmaceutically acceptable carrier.

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5. A method for treating and/or preventing asthma, allergy, general inflammation or cancer, said method comprising the step of administering an effective amount of an oligonucleotide as defined in claim 1, 2 or 3, to a patient in need of such a treatment.

6. A pharmaceutical composition comprising at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors and at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors.

7. The pharmaceutical composition according to claim 6, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

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8. The pharmaceutical composition according to claim 6, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

9. The pharmaceutical composition according to claim 6, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16 and at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

10. The pharmaceutical composition according to claim 6, further comprising at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

11. The pharmaceutical composition according to claim 10, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

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12. A pharmaceutical composition comprising at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

13. The pharmaceutical composition according to claim 12, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

14. The pharmaceutical composition according to claim 12, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

15. The pharmaceutical composition according to claim 12, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16 and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

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16. A pharmaceutical composition comprising at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

17. The pharmaceutical composition according to claim 16, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

18. The pharmaceutical composition according to claim 16, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

19. The pharmaceutical composition according to claim 16, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

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20. A method of treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, the method comprising administering to a patient at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors and at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors.

21. The method according to claim 20, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

22. The method according to claim 20, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

23. The method according to claim 20, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16; and at least one oligonucleotide directed

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against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

24. A method of treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, the method comprising administering to a patient at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors, at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors, and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

25. The method according to claim 24, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

26. The method according to claim 24, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

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27. The method according to claim 24, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

28. The method according to claim 24, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16; at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, and SEQ ID NO:7; and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO: 20, SEQ ID NO:22, and SEQ ID NO:23.

29. A method of treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, the method comprising administering to a patient at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

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30. The method according to claim 29, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

31. The method according to claim 29, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

32. The method according to claim 29, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16; and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

33. A method of treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, the method comprising administering to a patient at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and

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IL-13 receptors and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

34. The method according to claim 33, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

35. The method according to claim 33, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

36. The method according to claim 33, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7; and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

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REMARKS

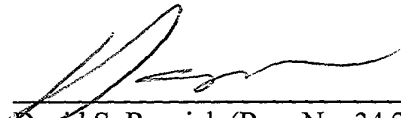
Support for the new claims can be found in the claims as originally filed. No new matter has been added and their entry is respectfully requested.

CONCLUSION

If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (617)-345-6057.

Respectfully submitted,
NIXON PEABODY LLP

Dated: December 15, 2000



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ANTISENSE OLIGONUCLEOTIDES FOR TREATING OR PREVENTING
ATOPIC DISEASES AND NEOPLASTIC CELL PROLIFERATION

BACKGROUND OF THE INVENTION

5 (a) Field of the Invention

The invention relates to the use of antisense oligonucleotides directed against specific cellular receptors, alone or in combination, in order to inhibit the inflammatory reaction that is present in asthma,
10 hypereosinophilia or atopic diseases and to inhibit neoplastic cell proliferation.

(b) Description of Prior Art

Antisense oligonucleotides are a new class of pharmaceuticals. In general, antisense refers to the
15 use of small, synthetic oligonucleotides, with the same constituents as that found in our own DNA and which resemble single stranded DNA. The antisense oligonucleotides are designed as a mirror sequence of a part of a gene they are targeting in order to be able
20 to adhere to this sequence and inhibit gene expression. Gene expression is inhibited through hybridization of sense oligonucleotide to a specific messenger RNA (mRNA) sense target according to the Watson-Crick base pairing in which adenosine and thymidine or guanosine
25 and cytidine interact through hydrogen bonding. These simple base-pairing rules govern the interaction between the antisense oligonucleotides and the cellular RNA, which allow to design an antisense oligonucleotide. A major advantage of this new
30 strategy is the specificity of action with the potential for less side effects and toxicity. This therapeutic strategy could potentially be applied to any disease where an overexpression of one or several genes is believed to cause the presence or persistence
35 of the disease. As a result, there have been numerous

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studies of antisense oligonucleotides as therapeutic agents for cancer and viral diseases.

Few studies have been performed in order to assess whether antisense oligonucleotides could inhibit receptor expression on cell surfaces for inflammatory mediators.

Antisense oligonucleotides can be used to inhibit interleukin (IL)-6 receptor expression and thus the effects of the acute inflammatory mediator interleukin-6 on cells. No studies have been conducted to assess whether antisense oligonucleotides can be employed to inhibit receptors on cells that are involved in asthmatic inflammation or on cancerous cells.

International Application published as WO9622371A (Ponath Paul D.) describes the C-C chemokine receptor CKR-3. It is the equivalent of the CCR3 receptor. In the application, there is no preliminary information on whether antisense constructs are effective at inhibiting the CCR-3 receptor. In addition there is no information on the fact that they are effective.

Ikizawa et al. "Inhibition of IL-4 receptor up-regulation on B cells antisense oligodeoxynucleotide suppresses IL-4-induced human IGE production" *Clinical and Experimental Immunology*, Vol. 100, No. 3, pp. 383-389, (1995)", describes antisense oligonucleotides against the IL-4 receptor. There is no mention that this is against the common IL-4 IL-13 receptor. There is no discussion of the common effects with IL-13 or of synergy with IL-13. There is also no discussion of the need to synergize by adding with other oligos.

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International Application published as WO 97 20926, identified the IL-13 receptor alpha and beta sequences. They mention the term antisense oligonucleotides without providing any further information on any sequence or data.

International Application published as WO 9728190 describes the common Beta c receptor for IL-3, IL-5 and GM-CSF. There is no description of the use of antisense oligos for the therapy of allergy, asthma or cancer in any part of the application.

International Application published as WO 97 23244 announces that ODNs are provided which are targeted to the nucleic acid encoding receptor negative regulatory domains. They are targeting genes that encode negative regulatory domains. They are looking at EPO.

International Application WO 97 41154 describes the eosinophil eotaxin receptor that has been designated "CC CKR3". Nowhere in the application is there any sequence or evidence of data for antisense oligonucleotides which can bind to eosinophil eotaxin receptor nucleotides and modulate receptor function or expression".

Ponath PD et al. "Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils" *Journal of Experimental Medicine*, Vol. 183, June 1996, pp. 2437-2448, (June 1996), only presents the eotaxin receptor CCR3 sequence.

International Application published as WO9741225A disclose polynucleotides against chemokine receptors for screening. Antisense ODNs are presented

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against the MMLR-CCR or MPHG-CCR chemokine receptors and do not correspond to the receptors of the present invention.

Asthma is a disease that affects 5 to 10% of the population which has doubled in prevalence in the last 25 years. This increase has been noted especially in infants after a viral infection of the airways (bronchiolitis), in children and in occupational induced asthma. The exact cause of asthma is not yet known. However, it is believed that agents such as viruses are involved in the perpetuation of the abnormal inflammation that is found in the airways of patients with asthma and thus the persistence of the disease.

For this reason the current recommendations for first line therapy of asthma is a potent anti-inflammatory medication such as corticosteroids and antileukotrienes. Although this therapy is effective in many patients, some patients are resistant to corticosteroids. This medication is also a potent immunosuppressive with long term side effects and has not been shown to be effective in the prevention of allergy or asthma.

Antileukotrienes have some effect in allergy and asthma but are not as effective as corticosteroids.

Several inflammatory mediators play a role in the appearance and perpetuation of inflammation in the
5 airways of patients with asthma. Some mediators attract the inflammatory cells into the airways either through chemotaxis of eosinophils (the chemokines: RANTES, eotaxin 1,2, MCP-3,4 that act mostly in asthmatic inflammation through a receptor called CCR3)
10 or through endothelial cell activation (IL-4,13). Other mediators cause the priming and increased survival of inflammatory cells in the airways (IL-3,5, GM-CSF, IL-4). These mediators thus consist of either specific chemokines for eosinophils or of cytokines of
15 the T helper lymphocyte type 2 phenotype (Th2: IL-3,4,5,13 and GM-CSF).

An improvement in asthma has been shown when there is a decrease in these inflammatory mediators in the airways.

20 Allergy is a disease that is extremely prevalent, for example atopic rhinitis affects around 30% of the population. Allergy is characterized by abnormal IgE production and inflammation to an allergen. In the presence of IgE and allergen,
25 effector cells such as the mast cells degranulate and release inflammatory mediators leading to the recruitment of the same inflammatory cells that are found in asthma. In atopic rhinitis, nasal polyposis and chronic sinusitis one finds the same excess in
30 inflammatory mediators as those present in asthma. IL-4 and IL-13 are necessary for the production of IgE and the induction of the cells with a Th2 phenotype.

Cancer is the second cause of death in humans and is characterized by abnormal proliferation of
35 immortalized cells. One of the mechanisms that is

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involved in the persistence and increase in these cells is by the release of growth factors that act through receptors and lead to cellular proliferation. Amongst these growth factors, GM-CSF has been shown to be an
5 important growth factor for several tumor cells. The inhibition of proliferation of cancerous cells by blocking the receptors for growth factors could be important in the therapy of certain cancers.

It would be desirable to be provided with the
10 use of antisense oligonucleotides directed against at least one specific common receptor for either Th2 cytokines or receptor for mediators that attract cells that respond to Th2 cytokines, in order to inhibit the inflammatory reaction that is present in asthma or
15 atopy and to inhibit neoplastic cell proliferation.

It would also be highly desirable to be provided with antisense oligonucleotides directed against a nucleic acid sequence coding for receptors so that by inhibiting these receptors these oligonucleotides could
20 be employed in the therapy and/or prevention of asthma, allergy, general inflammation and cancer.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide
25 the use of antisense oligonucleotides directed against at least one common subunit of a cellular receptor, such as the common beta subunit for IL-3, IL-5, and GM-CSF, or at least one of the common subunits for the IL-4 and IL-13 receptors or the receptor CCR3, in order to
30 inhibit the inflammatory reaction that is present in asthma or atopy and to inhibit neoplastic cell proliferation.

Another aim of the present invention is to provide antisense oligonucleotides directed against a
35 nucleic acid sequence coding for a common subunit of

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the IL-4 and IL-13 receptors so that by inhibiting these receptors these oligonucleotides could be employed in the treatment and/or prevention of asthma, allergy, general inflammation or cancer.

5 Another aim of the present invention is to provide antisense oligonucleotides directed against a nucleic acid sequence coding for the common beta subunit of the IL-3, IL-5 and GM-CSF receptors so that by inhibiting these receptors they may be employed in
10 the treatment and prevention of asthma, allergy, hypereosinophilia, general inflammation or cancer.

Another aim of the present invention is to provide antisense oligonucleotides directed against a nucleic acid sequence coding for the CCR3 receptor for
15 chemokines so that by inhibiting this receptor they may be employed in the treatment and prevention of asthma, allergy, general inflammation or cancer.

Another aim of the present invention is to provide a therapeutically effective composition
20 comprising at least two antisense oligonucleotides directed against nucleic acid sequences coding for the common subunits of IL-4 and IL-13 or the common beta subunit of IL-3, IL-5, and GM-CSF, or the CCR3 receptors for a more potent effect in the treatment
25 and/or prevention of asthma, allergy, general inflammation or cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates increased IL-4 production in
30 response to the house dust-mite antigen in subjects with early wheezing;

Figs. 2A and 2B illustrate cytokine production during bronchiolitis as a predictor of the severity of wheezing;

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Figs. 3A and 3B illustrate the relationship between IFN production in response to IL-2 by blood mononuclear cells and the development of asthma 2 years after bronchiolitis in infants;

5 Figs. 4A and 4B illustrate the correlation between interferon gamma production in response to IL-2 at the time of bronchiolitis in infants and Vmax FRC (4A) or PC40 histamine (4B);

10 Figs. 5A to 5C illustrate the distribution of an FITC labeled antisense phosphorothioate oligonucleotide 8 hours after being nebulized or breathed into the lungs of a rat;

15 Figs. 6A and 6B illustrate inflammatory cells (Fig. 6A) and an FITC-labeled antisense phosphorothioate oligonucleotide which has found its way into the inflammatory cells (green fluorescence) (Fig. 6B) retrieved from lung lavage of rats 24 hours after administration;

20 Fig. 7 illustrates a gel showing the antisense phosphorothioate oligonucleotides still intact when retrieved from the bronchoalveolar lavage (BAL) and from the lungs (biopsies) of rats 24 hours after administration when compared to a control antisense oligonucleotide (Eot.FITC);

25 Fig. 8 illustrates the antisense phosphorothioate oligonucleotides OD1, OD2 and OD3 inhibiting IL-4 and IL-13 receptor expression in RAJI cells as detected by Flow cytometry;

30 Figs. 9A and 9B illustrate the antisense phosphorothioate oligonucleotides OD1, OD2 and OD3 in accordance with one embodiment of the invention, inhibiting protein expression of the IL-4 receptor in RAJI cells as detected by immunoprecipitation and Western;

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Fig. 10 illustrates the antisense phosphorothioate oligonucleotide A1.3 inhibiting mRNA expression of the alpha' sub-unit of the IL-4/13 receptors;

5 Figs. 11A to 11F illustrate the dose response of the antisense oligonucleotide OD2 at inhibiting protein expression of the IL-4 and IL-13 receptor in RAJI cells as detected by immunocytochemistry;

10 Fig. 12 illustrates the antisense phosphorothioate oligonucleotides OD2 and A1.3 inhibiting IL-4 and increasing IFN- γ production in human umbilical cord blood cell Th-2 progenitor cells;

15 Figs. 13A and 13B illustrate the antisense phosphorothioate oligonucleotide 107A inhibiting mRNA expression (by semi-quantitative RT-PCR) of the common beta sub-unit of the IL-3, IL-5 and GM-CSF receptor in TF1 (Fig. 13A) and U937 (Fig. 13B) cells;

20 Fig. 14 illustrates the antisense phosphorothioate oligonucleotide 107A inhibiting protein expression of the common beta sub-unit of the IL-3, IL-5 and GM-CSF receptors in TF1 cells as detected by immunoprecipitation and Western;

25 Fig. 15 illustrates the antisense phosphorothioate oligonucleotide 107A inhibiting as a dose response TF1 cell proliferation;

Fig. 16 illustrates the antisense phosphorothioate oligonucleotide 107A inhibiting TF1 cell growth;

30 Fig. 17 illustrates the antisense phosphorothioate oligonucleotide RB141A (also referred to as AS141) inhibiting mRNA expression of the beta sub-unit of the GM-CSF, IL-3 and IL-5 receptors in rat bone marrow cells;

35 Figs. 18A and 18B illustrate the antisense phosphorothioate oligonucleotides RB141A (directed

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against the beta sub-unit of the rat GM-CSF, IL-3 and IL-5 receptors), RCC3A4 (directed against the rat CCR3 receptor) or a combination of both inhibiting eosinophil influx into the peritoneal cavity (left) or the lungs (right) after ovalbumin challenge;

Figs. 19A to 19H illustrate the expression and cellular distribution of eotaxin mRNA (Figs. 19A to 19D) and protein (Figs. 19E to 19H) in airways (Figs. 19A, 19B, 19E) and BAL cells (Figs. 19C, 19D, 19F to 19H) of allergic asthmatic patients (Figs. 19A, 19C, 19E, 19F, 19G) and normal controls (Figs. 19B, 19D, 19H);

Fig. 20 illustrates the effect of the antisense directed against the CCR3 receptor (RC86A, called in the figure: CCR3AS 86) on CCR3 mRNA expression in HL60 cells (clone 15) that have been differentiated into the eosinophil pathway;

Fig. 21 illustrates the effect of the antisense RC86A directed against the CCR3 receptor on CCR3 mRNA expression in Ghost cells transfected with the CCR3 receptor;

Fig. 22 illustrates the effect of the phosphorothioate antisense RC86A and the phosphorothioate antisense RC 269 directed against the CCR3 receptor on calcium mobilization in purified human eosinophils;

Fig. 23 illustrates the effect of the phosphorothioate antisense oligonucleotide RCC3A4 directed against the rat CCR3 receptor on CCR3 mRNA expression in rat bone marrow cells;

Fig. 24 illustrates the effect of preincubation with IL-5 on the chemotaxis induced by eotaxin; and

Figs. 25A to 25I illustrate the effect of preincubation with IL-5 overnight on chemokine production.

DETAILED DESCRIPTION OF THE INVENTION

Bronchiolitis is a viral infection of the
airways of infants that predisposes to the development
of asthma. This condition was studied since it is the
earliest one can get in humans prior to the development
of asthma, atopy, and allergic inflammation. As is
shown hereinafter, an imbalance in the Th1 to Th2
cytokine ratio, favoring Th2 cytokines, is present
prior to developing asthma. In one embodiment, the
present invention is aimed at restoring this imbalance
and thus at preventing or treating asthma and
allergies.

Results obtained in lymphocytes isolated from
infant blood suffering from bronchiolitis have
suggested and confirmed that an imbalance exists
between Th1 and Th2 cytokine production prior to the
development of early wheezing. Indeed, Fig. 1 shows
that lymphocytes from infants who wheeze after
bronchiolitis have an increased production of IL-4 (a
Th2 cytokine) after exposure to the house dust-mite
antigen. In Fig. 1, lymphocytes were isolated from the
blood of infants 5 months after bronchiolitis and
cultured in the presence of the house dust-mite
antigen. IL-4 was measured in the supernatant
collected 3 days after culture. Results are presented
for the subjects who wheezed for at least one of the
last 90 days and those who did not wheeze at all within
the first 5 months after bronchiolitis. Asterisk
indicates subject who had an IL-4 level of 535 pg/ml.
In addition, in infants that wheezed the most in the
first five months after bronchiolitis a lower
interferon gamma (IFN, a Th1 cytokine) production and a
higher IL-4 production was found.

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The condition of these infants were monitored for 2 years, pursuant to which it was determined whether they had no asthma, possible asthma or probable asthma by the Delphi consensus. The smoking history and the presence of atopy or asthma in parents or siblings were recorded and blood mononuclear cell IFN and IL-4 production in response to IL-2 were assessed in 32 infants hospitalized for bronchiolitis and in a sub-group (n=19) in which pulmonary function tests were performed 4.9 months later.

In Figs. 2A and 2B, lymphocytes were isolated from subjects during bronchiolitis and cultured in the presence of IL-2 for 3 days. The supernatant was collected and the cytokines measured by ELISA. Results are presented for the subjects who wheezed more than 20 days (more wheezing, n=9) and those who wheezed for fewer than 20 days (less wheezing, n=6).

Infants with possible and probable asthma had lower IFN production at the time of, and 4.9 months after bronchiolitis when compared to those who had no asthma ($p < 0.05$, Figs. 3A and 3B).

In Figs. 3A and 3B, mononuclear cells obtained at the time of bronchiolitis (3A, n=32) or 4.9 months later (3B, n=19) were partially depleted of monocytes and cultured with IL-2 for 3 days. The supernatant was retrieved and IFN production was measured by ELISA. Results are presented for patients evaluated 2 years after bronchiolitis as having no asthma (no), possible asthma (possible) and probable asthma (probable). For results identified by "*", a probability of $p < 0.05$ was found using the Kruskal-Wallis test and Mann-Whitney U test possible and probable versus no asthma. For results identified by "***", a probability of $p = 0.08$ was found using the Kruskal-Wallis. For results identified by "++", a probability of $p < 0.05$ was found

using the Mann-Whitney U test possible and probable asthma versus no asthma.

IL-4 production did not differ between groups. Significant positive correlations were found between IFN production at the time of bronchiolitis and markers of abnormal airway function (Vmax of functional residual capacity (FRC), Fig. 4A)) or of increased airway responsiveness (PC40 histamine, Fig. 4B)), 4.9 months after bronchiolitis.

In Figs. 4A and 4B, cytokine production was measured at the time of bronchiolitis and pulmonary function was measured 4.9 months later. Pulmonary function was evaluated with methods recommended by the American Thoracic Society. Maximal expiratory flow at functional residual capacity (Vmax FRC) was assessed by the rapid thoracoabdominal compression technique (RTC) using the following procedure. Patients previously sedated with chloral hydrate 100 mg/Kg body weight (maximal dose 1000 mg) were placed supine with the neck slightly extended in an inflatable jacket covering the abdomen and thorax and connected to a pressure reservoir. Starting from a pressure of 30 cm H2O and using increments of 5 cm H2O, measurements of expiratory flow at FRC were obtained until Vmax FRC was achieved. Flows were measured with a soft cushion mask connected to a Fleisch no. 1 pneumotachograph and integrated. Three additional technically correct maneuvers were performed at this pressure from which the highest value was chosen to represent baseline Vmax FRC. All subsequent Vmax FRC maneuvers were carried out using the same procedure.

Bronchial reactivity to histamine was assessed by using a Hudson updraft #2 nebuliser driven at 8 liters/min. to administer doubling concentrations of histamine starting at 0.0625 mg/ml to a maximum of 8.0

mg/ml for 1 minute at 5 minute intervals. Vmax FRC was determined after each nebulization. The challenge test ended when a decrease in Vmax FRC of at least 40% from baseline value had been reached, or the maximum concentration of histamine had been given. Heart rate and oxygen saturation were continuously monitored throughout the study with an Ohmeda BIOX 3740 pulse oximeter.

A defect in IFN production is a primary contributor to the development of asthma in infants. Interestingly, this defect is present in adults with asthma and in newborns before they develop atopy. There thus is an imbalance in the relative production of Th2 (IL-4, IL-13, IL-5, etc.) vs. Th1 (IFN) cytokines that is present even before one develops asthma or allergy, the ratio of Th2 over Th1 cytokines is increased prior to the development of and during these diseases.

In order to treat or prevent the development of allergy, asthma or neoplastic cell proliferation that is dependent on an abnormal increase in the production or the effects of Th2 cytokines, it was thus found desirable to decrease the effects of the Th2 cytokines.

Accordingly, there is provided hereinafter evidence that antisense oligonucleotides according to one embodiment of the present invention, which are breathed into the lungs, are deposited therein, and enter cells where they are active and remain in a non-degraded and thus potent state for at least 24 hours (See Fig. 5 and Example I).

Antisense oligonucleotides according to a preferred embodiment of the present invention are directed against at least one common sub-unit of the IL-4 and IL-13 receptors. These antisense oligonucleotides are effective at inhibiting the

functional sub-units of these receptors, as illustrated in Example II.

Antisense oligonucleotides in accordance with another embodiment of the present invention are directed against the common beta sub-unit of the IL-3,5 and GM-CSF receptors. These antisense oligonucleotides are effective at inhibiting these receptors and thus at preventing the proliferation or function of cancerous or inflammatory cells that depend on these growth factors for survival (See Example III).

Antisense oligonucleotides in accordance with another embodiment of the present invention are directed against the CCR3 receptor of chemokines. These antisense oligonucleotides are effective at inhibiting this receptor and thus at preventing the influx, survival and proliferation or function of inflammatory cells and cancerous cells or infectious organisms that depend on this receptor (See Example IV).

The present invention will be more readily understood by referring to the following examples which are given to illustrate the following invention rather than to limit its scope.

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EXAMPLE I

Effective administration of antisense oligonucleotides

In order for any therapy to be effective, the administered substance must first find it's way into the lungs and to the cells where it is to have its effects and second, to remain intact without having any side effects. Antisense oligonucleotides breathed into the lungs, are deposited in the lungs and airways to enter the cells where they have their effects and remain in a non-degraded state for at least 24 hours without affecting lung physiology. One microgram (1 μ g) of antisense phosphorothioate oligonucleotide of

the present invention that had previously been tagged with FITC was administered by nebulization into the lungs of rats. Rats were anesthetized with urethane (1 g/kg, i.p.). A heating pad was used to maintain body temperature constant during the experiment and rectal temperature was monitored continuously with an electronic thermometer. After blind orotracheal intubation with 6 cm of PE-240 polyethylene catheter, pulmonary resistance was measured during spontaneous tidal breathing with the animals in the supine position. Flow was measured by placing the tip of the tracheal tube inside a small Plexiglas® box (265 ml in volume). A Fleisch no. O pneumotachograph coupled to a piezoresistive differential pressure transducer (Micro-Switch 163PCOID36, Honeywell, Scarborough Ont. Canada) was attached to the other end of the box to measure airflow. Transpulmonary pressure (Ptp) was measured using a water-filled catheter placed in the lower third of the esophagus connected to one port of a differential pressure transducer (Transpac II, Abbott, Illinois), the other port being connected to the Plexiglas box. The esophageal catheter consisted of a polyethylene tube (PE-240, 10 cm long) with a terminal tip (6 cm) of a smaller bore tube (PE-160).

The pressure and flow signals were amplified, passed through eight-pole Bessel filters (9 model 902LPF, Frequency Devices, Haverhill, MA) with their cut off frequencies set at 100 Hz. The data were stored on a computer. Lung resistance was calculated by multiple linear regression by fitting the equation of motion as performed with commercial software (RHT Infodat Inc. Montreal, PQ).

After instrumentation, an aerosol of saline containing 1 μ g of the tagged phosphorothioate oligonucleotide was administered for five minutes.

This was generated using a Hudson nebulizer with an output of 0.18 ml/min. connected to one side port of the box. The box was flushed with a stream of fresh air between measurements in order to prevent the accumulation of CO₂. Lung resistance was measured 5, 10, 15, 20 and 30 minutes after challenge and subsequently every 15 minutes for a total time of 8 hours. Lung resistance did not change over this time period. The rats were then killed by exsanguination and the lungs retrieved to determine whether the oligonucleotide was still present. The lungs were fixed in paraformaldehyde and an anti-FITC antibody tagged with alkaline phosphatase was used to determine the site of the oligonucleotide, the tissue samples were revealed with fast red and the nucleus of the cells counterstained with a Hoechst counterstain. It is to be noted in Fig. 5A that the oligonucleotides (in red) are present diffusely in all cell types. The oligonucleotides have penetrated the cytoplasm of the cells (5B) and are also found in an inflammatory cell (macrophage, in the middle of 5C).

In other experiments, the rats were anesthetized with pentothal and awakened after antisense nebulization. Bronchoalveolar lung lavage (BAL) was performed 24 hours later after general anesthesia by administration of 5 ml of saline and gentle aspiration. The BAL was centrifuged at 400xg for 10 minutes, the supernatant frozen and the cells centrifuged onto slides for analysis. It is to be noted in Fig. 6A that macrophages are the predominant cell type. The FITC-labeled oligonucleotide (green fluorescence) in Fig. 6B is present in the cytoplasm of the cells. The FITC-labeled oligonucleotide was either extracted from the lavage or the lungs of the rats 24 hours after antigen challenge. It is to be noted in Fig. 7 that the

antisense oligonucleotide is also intact 24 hours after administration in the BAL (lane 1), lung (lane 2) when compared to it's own control (lane 3) or another oligonucleotide that is tagged with FITC (eotaxin, lane 4).

As can be shown from Figs. 5 to 7, the antisense oligonucleotides of the present invention are breathed into the lungs, to penetrate the cells, remaining intact for more than 24 hours.

EXAMPLE II

Antisense oligonucleotides inhibiting the common sub-units of the IL-4 and IL-13 receptors

Interleukin-4 is involved in IgE production, the development and persistence of asthma and atopy. Although therapies directed against the effects of IL-4 may be effective in the prevention of asthma, allergy or neoplastic cell proliferation (that depends on this mediator), it has recently been shown that another Th2 cytokine (IL-13) has the same effects as IL-4. Interestingly IL-4 and IL-13 share at least two common sub-units which are necessary for signal transduction of the message to occur.

Experiments were performed to assess whether antisense oligonucleotides directed against the common sub-units of the IL-4 and IL-13 receptors could inhibit the expression of these receptors. RAJI cells express high levels of IL-4 and IL-13 receptors. These cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin, streptomycin and l-glutamine at 37°C in 5% CO₂. For 12 hours the cells were either cultured in medium alone or medium with sense or antisense oligonucleotides to the common sub-unit of IL-4/IL-13. The cells were retrieved, washed 3 times and then stained with an anti-human IL-4 receptor antibody (R and D systems, catalog number

MAB230), which has been shown to block the human cell surface receptor-mediated bioactivities caused by IL-4 or IL-13. It is to be noted in Fig. 8 that the antisense oligonucleotide OD1: 5'-agaccttcat gttcccagag-3' (SEQ ID NO:1), OD2: 5'-gttcccagag cttgccacct-3' (SEQ ID NO:2) or OD3: 5'-cctgcaagac cttcatggtt-3' (SEQ ID NO:3) inhibits the expression of the bioactive form of the IL-4 receptor when assessed by flow cytometry. The first line shows the absence of fluorescence in cells that were either unstained (left) or exposed to a non-specific monoclonal antibody (right). The second line shows that RAJI cells express the IL-4 receptor (92%) and that the fluorescence intensity is very high (many receptors). On the right, the RAJI cells were incubated for 12 hours with 10 μ Mol of the antisense oligonucleotide OD1 showing that only 66% of the cells express this receptor and that the fluorescence intensity is very low (few receptors on each cell). The third line shows the same results after 12 hours of incubation with the antisense oligonucleotides OD2 (52%) and OD3 (58%).

Additional experiments were performed to assess whether antisense oligonucleotides (OD3, OD2 and OD1) inhibited IL-4 receptor expression on RAJI cells by immunoprecipitation and Western blotting. It is to be noted in Fig. 9 that thirty million RAJI cells were cultured for 12 hours as previously described in complete medium with either 20 μ M of the sense oligonucleotide OD4 (first lane from the left), 10 μ M of OD4 (second lane from the left), 10 μ M of the antisense oligonucleotide OD3 (third lane from the left), 10 μ M of the antisense oligonucleotide OD2 (fourth lane from the left), 10 μ M of the antisense oligonucleotide OD1 (fifth lane from the left), medium alone (sixth lane from the left and last lane on the right of the second gel),

20μM of the antisense oligonucleotide OD2 (first lane from the left of the gel on the right). The total protein was extracted and incubated with 2 μg of IL-4 overnight. Ten (10) μg/ml of anti-IL-4 antibody (R and D systems) coupled to 50 μl of protein A and Protein G-Sepharose™ was then added for two (2) hours at 20°C. The Sepharose™ beads were washed ten times and an agarose gel was used to separate remaining proteins. The remaining proteins were then transferred onto an Immobilon-P-millipore™ membrane and the Western revealed by a rabbit polyclonal anti-IL-4-R-alpha antibody (Santa Cruz biotechnology, Inc., cat# sc-684). The results show that sense oligonucleotides do not affect IL-4 receptor expression, that 10μM of the effective antisense oligonucleotides of the present invention inhibit IL-4 receptor expression and that 20μM of the antisense oligonucleotide OD2 is almost completely effective.

Since there are at least 2 (two) sub-units that are common to the IL-4 and IL-13 receptors additional experiments were performed to determine whether antisense oligonucleotides directed against the alpha' chain of the IL-13 receptor would also inhibit IL-4/13 receptor expression on RAJI cells. It is to be noted in Fig. 10 that the antisense oligonucleotide A1.3: 5'-CGCCCACAGC CCGCAGAGCC-3' (SEQ ID NO:4) inhibited alpha' receptor expression in RAJI cells. RAJI cells were incubated for 3 hours in medium containing antisense (10 or 20 μM) or sense oligonucleotides (10μM) and then for 9 hours in complete medium. Thereafter mRNA was extracted. IL-13Ralpha' chain, and controls IL-2Rgamma chain and G3PDH transcripts were assessed by semiquantitative RT-PCR. Results are presented from left to right for peripheral blood mononuclear cells untreated (PBMNC, lane 2), RAJI cells in complete

medium (lanes 3, 4), RAJI cells with 10 μ M of A1.3 (lane 5), RAJI cells with 10 μ M of A1.3 and its sense counterpart (S1.3, lane 6), RAJI cells with 20 μ M of A1.3 (lane 7), and no cDNA (lane 8). Sequences of other antisense oligonucleotides that were also effective *in vitro* include: A1.1: 5'-CTCCATGCAG CCTCTCGCCT-3' (SEQ ID NO:5); A1.4: 5'-CCGCCGGCGC AGAGCAGCAG-3' (SEQ ID NO:6); and A1.5: 5'-CGCCCCCGCC CCCGCCCCCG-3' (SEQ ID NO:7).

Dose response experiments were performed with the antisense oligonucleotide OD2 to determine the optimal concentration that block IL-4/IL-13 receptor expression in RAJI cells. It is to be noted in Fig. 10 which shows immunostaining experiments that OD2 also inhibited receptor expression when assessed by immunostaining studies. RAJI cells were cultured for 12 hours in complete medium containing 5 μ M OD2 (upper left), 10 μ M OD2 (middle left), 20 μ M OD2 (lower left), no oligonucleotide (upper right), 10 μ M of the sense oligonucleotide for the same sequence as OD2 (middle right) or 20 μ M of the sense oligonucleotide for the same sequence as OD2 (lower right). Slides were fixed in methanol-acetone at -20°C for 10 min. After treatment with Tris-buffered saline containing universal blocking solution (DAKO) for 15 min., slides were incubated with an anti-IL-4 receptor serum (Santa Cruz biotechnology, Inc., cat# sc-684) at a final dilution of 1/200 overnight at 4°C., followed by incubation with 5 μ g/ml alkaline phosphatase-labeled goat anti-rabbit IgG. Nuclei of cells were stained for 1 min. in Haematoxylin. Under these experimental conditions 20 μ M of OD2 almost completely inhibited IL-4 receptor expression.

IgE antibody production is an important component of allergy, asthma and certain neoplastic

conditions. Experiments were performed to assess the effect of antisense phosphorothioate oligonucleotides directed against the alpha (OD2) and alpha' (A1.3) common sub-units of the IL-4 and IL-13 receptors on IgE production. Human B lymphocytes were isolated from tonsils surgically removed for a variety of indications. Mononuclear cells were purified by Ficoll-Hypaque™ (Pharmacia) density centrifugation. B lymphocytes were separated from T cells by E-rosetting with neuraminidase treated (Calbiochem. La Jolla, Calif.) sheep red blood cells. Monocytes were adherence depleted on plastic petri dishes for 2 hours. B lymphocytes purity was routinely 98%. B cells (2 x 10⁵/200 µl/well) were cultured in complete medium consisting of RPMI 1640, supplemented with 10% FCS, 10 mg/ml L-glutamine, 50 U/ml penicillin, and 50 ng/ml streptomycin. The cells were stimulated with a combination of 200 U/ml of IL-4 (R&D System) and 0.1 µg/ml of anti-CD40 mAb (Pharmlngen) in the presence or absence of antisense or sense oligonucleotides at 10 µM for 14 days. Supernatants were harvested and IgE was measured by ELISA (Kallestad, Sanofi Diagnostics, Chaska, Minn.) according to the manufacturer's instructions. It is to be noted in Table 1 that both A1.3 and OD2 antisense phosphorothioate oligonucleotides inhibit human IgE production when compared to their sense counterparts.

Table 1
Inhibition of IgE production by antisense
oligonucleotides in anti-CD40 + IL-4
stimulated human B cells

| | IgE in pg/ml |
|----------------------------------|--------------|
| Medium | <200 |
| Anti-CD40 + IL-4 | 1109 |
| + A1.3 (antisense 10 μ M) | 122 |
| + A1.3 (strand sense 10 μ M) | 1001 |
| + OD2 (antisense 10 μ M) | 364 |
| + OD2 (strand sense 10 μ M) | 1039 |

As mentioned previously, an imbalance between production of the Th-2 cytokine interleukin-4 and the Th-1 cytokine IFN- γ has been described in allergy and asthma. Experiments were performed to assess the effect of antisense phosphorothioate oligonucleotides directed against the alpha (OD2) and alpha' (A1.3) common sub-units of the IL-4 and IL-13 receptors on IL-4 and IFN- γ production. Human mononuclear cell suspensions were obtained from umbilical cord blood (UCB) by centrifugation over Ficoll-Hypaque™ gradient at 400g for 30 minutes. Monocytes were depleted by adherence to plastic flasks during 2 hours at 37°C in RPMI 1640 supplemented with 7.5% fetal calf serum (FCS) in a 5% CO₂ incubator. Cord blood T cells were stimulated with PHA (1 μ g/ml) under TH1 polarizing conditions [human rIL-12 (2 ng/ml; R&D Systems) plus neutralizing mAb to IL-4 (200 ng/ml; R&D Systems)] or TH2 polarizing conditions [human recombinant IL-4 (200 U/ml) plus neutralizing mAb to human IL-12 (2 μ g/ml; R&D Systems)] in the presence or absence of 10 μ M of antisense or sense oligonucleotides. Each oligonucleotide was added to the culture 3 hours before the addition of the cytokines. IL-2 was added at day 3. After 1 and 2

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weeks, the cultures were restimulated in the same polarizing conditions in the presence or absence of oligonucleotides and analyzed after 10 days of culture. Cells were harvested, washed three times and
5 restimulated with PMA (50 ng/ml) plus ionomycin (500 ng/ml) for 48h at 37°C, for analysis of IL-4 and IFN- γ production in supernatants by ELISA (R&D Systems) according to the manufacturer's instructions. It is to be noted in Fig. 12 that antisense oligonucleotides
10 directed against the common sub-units of the IL-4/13 receptors inhibit IL-4 production and increase IFN- γ production. From left to right, purified UBC were cultured in Th-2 stimulating conditions: Th-2 stimulating conditions in the presence of the sense oligonucleotide for OD2; Th-2 stimulating conditions in
15 the presence of the antisense oligonucleotide OD2 and Th-2 stimulating conditions in the presence of the antisense oligonucleotide A1.3.

As can be shown from Figs. 8 to 12, the
20 antisense oligonucleotides of the present invention directed against the common sub-units alpha and alpha' of the IL-4/IL-13 receptor are effective at inhibiting IL-4/13 receptor expression, their functional component, IgE production and the switching of
25 progenitors to Th-2 type cells.

EXAMPLE III

Antisense oligonucleotides inhibiting the common beta sub-unit of IL-3, IL-5 and GM-CSF receptors

30 Interleukin-3, 5 and GM-CSF are important cytokines that are involved in eosinophil proliferation and survival. These cytokines are increased in asthma and atopic diseases and are also involved in the indefinite proliferation of certain neoplastic
35 diseases. Interestingly, IL-3, IL-5 and GM-CSF share a

common beta sub-unit that is involved in signal transduction.

Experiments were performed to assess whether antisense oligonucleotides of the present invention, directed against the common beta sub-unit of the IL-3, IL-5 and GM-CSF receptor, could inhibit the expression and the function of this receptor. TF-1 and U937 cells express high levels of GM-CSF receptors. In addition, TF-1 cells are dependent on GM-CSF for survival. These cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin, streptomycin and l-glutamine at 37°C in 5% CO₂ (the TF-1 cells were supplemented with GM-CSF). For 12 hours they were either cultured in medium alone or medium with sense (107S: 5'-ACCATCCCGC TGCAGACCC-3' (SEQ ID NO:8)) or antisense (107A: 5'-GGGTCTGCAG CGGGATGGT-3' (SEQ ID NO:9)) oligonucleotides to the common beta sub-unit of the IL-3, IL-5 and GM-CSF receptor. The cells were retrieved and washed 3 times. RNA was then retrieved and the presence of the beta chain of the receptor was assessed by semi-quantitative RT-PCR. It is to be noted in Fig. 13 that the antisense oligonucleotides inhibit the expression of mRNA for the common beta receptor in TF1 cells (13A) and U937 cells (13B). In Fig. 13A, read from left to right, mRNA expression for Beta actin is shown in control, sense, and antisense treated cells (lanes 2,3,4); mRNA expression for the common receptor is shown in control, sense and antisense treated cells (lanes 5,6,7). The absence of a band in lane 7 is indicative of the effectiveness of the antisense oligonucleotide at inhibiting mRNA expression of the common Beta sub-unit in TF1 cells. In Fig. 13B, read from the right to left, mRNA expression for the common beta sub-unit is shown in mismatch, antisense, sense

and control (non treated) cells (lanes 1,2,3,4); mRNA expression for G3PDH is shown in mismatch, antisense, sense and control (non treated) cells (lanes 6,7,8,9). The absence of a band in lane 2 is indicative of the effectiveness of the antisense oligonucleotide at inhibiting mRNA expression of the common Beta sub-unit in U937 cells.

Additional experiments were performed to assess whether antisense oligonucleotides (107A) inhibited the common beta sub-unit of IL-3, IL-5 and GM-CSF receptors in TF1 cells by immunoprecipitation and Western blotting. In Fig. 14, thirty million TF1 cells were cultured for 12 hours as previously described in complete medium with either 10 μ M of the sense oligonucleotide 107S or the antisense oligonucleotide 107A (first lane from the right). The protein was extracted by immunoprecipitation with a monoclonal antibody against the GM-CSF beta chain receptor. The extracts were then transferred onto an Immobilon-P-millipore membrane after electrophoresis on a polyacrylamide gel, and the GM-CSF beta chain of the receptor was then revealed by a rabbit polyclonal anti-GM-CSF-R-Beta antibody. The results show that, at the same concentration (10 μ M), sense oligonucleotides do not affect the common beta chain expression, while the antisense oligonucleotides of the present invention inhibit the common beta sub-unit of IL-3, IL-5 and GM-CSF receptors.

Dose response experiments were performed with the antisense oligonucleotide 107A to determine the optimal concentration that would block TF1 cell growth. As seen in Fig. 15, antisense oligonucleotides of the present invention can be used to inhibit cell growth. TF1 cells were cultured in the presence of increasing concentrations of the oligonucleotides in serum free

medium for 2 hours and then fetal bovine serum and GM-CSF were added to a final concentration of 10% and 1 ng/ml, respectively. The culture was performed for an additional 2 days and then cells were assayed for their capacity to reduce MTT dye over a four (4) hour period to a colored formazan product as an index of cell survival and proliferation. The results are expressed as a percentage of absorbance of MTT-derived formazan developed by untreated cells. Dot = mean \pm SDEV. The experiment was performed in triplicate. Absorbance was read at 570-595 nm.

It is to be noted in Fig. 16 that the antisense oligonucleotide 107A can significantly inhibit cell growth when compared to the sense probe or a control without antisense oligonucleotides. TF1 cells were cultured in the presence of the antisense oligonucleotide (first from right), the sense oligonucleotide (2nd from right), control medium (including GM-CSF, 3rd right) or medium without GM-CSF (4th from right) for 2 days and then cells were assayed for their capacity to reduce MTT dye as described above.

Other antisense oligonucleotides in accordance with the present invention have shown effectiveness at a concentration of 20 μ Mol. These antisense oligonucleotides are for example, but without limitation, the oligonucleotides 106: 5'-ggctctgcagc gggatgggtt-3' (SEQ ID NO:10); 108: 5'-agggtctgca gcgggatgg-3' (SEQ ID NO:11); 110: 5'-gcaggggtctg cagcgggat-3' (SEQ ID NO:12); 101: 5'-gcagcgggat ggtttcttc-3' (SEQ ID NO:13); 100: 5'-cagcgggatg gtttcttct-3' (SEQ ID NO:14); and 105: 5'-gtctgcagcg ggatggttt-3' (SEQ ID NO:15).

Experiments were performed to determine whether antisense oligonucleotides directed against the common beta chain of the GM-CSF, IL-3 and IL-5 receptors could

inhibit the influx of eosinophils *in vivo*. It is to be noted in Fig. 17 that the antisense oligonucleotide RB141A: 5'-TGGCACTTTA GGTGGCTG-3' (SEQ ID NO:16) is effective at inhibiting mRNA expression of rat common beta chain of the GM-CSF, IL-3 and IL-5 receptors *in vitro*. On the left are results of semi-quantitative RT-PCR for the G3PDH gene in control, RB141A and sense treated SS141 marrow cells. On the right, from right to left are the results of semiquantitative RT-PCR of the common beta sub-unit of the GM-CSF, IL-3 and IL-5 receptors; lane 2 shows the inhibition of gene expression with RB141A (AS 141). Brown Norway rats were anesthetized with pentothal (65 mg/Kg i.p.). They were then killed, lower extremities retrieved and femurs isolated. The bones were incubated in 70% ethanol for 10 minutes. The two extremities of the bones were sectioned and bone marrow obtained by injection and aspiration of 15 ml of RPMI 1640. The cells were washed twice and incubated for 6 hours in RPMI 1640 at 37°C in 5% CO₂ with medium alone, 10 μM RB141A or 10 μM of the sense oligonucleotide RB141S: 5'-CAGCCACCTA AAGTGCCA-3' (SEQ ID NO:17).

In additional experiments, Brown Norway rats were actively sensitized to ovalbumin by injecting 1.25 mg ovalbumin mixed with 200 mg aluminum hydroxide subcutaneously. On day 14, rats received 500 μg of RB141A in 500 μl of 0.9%NaCl intra-peritoneally. The next day, after general anesthesia with 65 mg/Kg pentothal and endotracheal intubation, 200 μg of the antisense is administered either intra-tracheally or intra-peritoneally in 50 μl of 0.9%NaCl. Twenty minutes later ovalbumin challenge is performed by injecting 200 micrograms of ovalbumin in 50 μl either intra-tracheally or intra-peritoneally. After 8 hours, the rats are again intubated after general anesthesia and a

lung lavage consisting of 5 times 5 ml instillations or a peritoneal lavage with 8 ml is performed. Cells are washed, counted and centrifuged onto slides in a Cytospin III. A differential cell count is performed.

5 It is to be noted in Fig. A that the antisense oligonucleotide RB141A directed against the common beta chain of the GM-CSF, IL-3 and IL-5 receptors inhibits eosinophil influx into the lungs or the peritoneal cavity by approximately 50%. In Fig. 18A are the results obtained from peritoneal fluid showing the effects of RB141 (AS141). In Fig. 18B are the results from lung BAL showing the effects of RB141 (AS141).

10 These results show that antisense oligonucleotides directed against the common beta chain of the GM-CSF, IL-3 and IL-5 receptors are important in the prevention of the eosinophil influx and/or survival that occurs in different allergic diseases.

15 As can be shown from Figs. 15 to 18, the antisense oligonucleotides of the present invention directed against the common beta sub-unit of the IL-3, IL-5 and GM-CSF receptors are effective at inhibiting receptor expression, cell growth, eosinophil recruitment and survival.

25 **EXAMPLE IV**
Antisense oligonucleotides inhibiting the CCR3 receptor
for chemokines

There are two (2) considerations with regards to the CCR3 receptor: 1) it is expressed on the Th2 and not on the Th1 lymphocytes, 2) the CCR3 receptor is important for the recruitment of eosinophils into the sites of allergic or asthmatic inflammation. The chemokines Eotaxin, MCP-4 and RANTES mediate most of their effects through the CCR3 receptor. These chemokines are present and increased in the lungs of patients with allergy and asthma (Lamkhioed et al.,

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Journal of Immunology, 159:4593-4601, 1997). Fig. 19 shows that eotaxin is increased in epithelial cells and inflammatory cells in the lungs of patients with allergy and asthma. The expression and cellular distribution of eotaxin mRNA (19A to 19D) and protein (19E to 19H) in airways (19A, 19B, 19E) and BAL cells (19C, 19D, 19F to 19H) of asthmatic patients and normal controls have been assessed. Eotaxin mRNA expression is increased in asthmatic (19A) compared with normal (19B) airways. Prominent staining is observed in epithelial cells (Ep) and in many inflammatory cells (arrowheads) of the allergic asthmatic airway. Figs. 19C and 19D are representative examples of *in situ* hybridization of cytopsin preparations of BAL cells obtained from an asthmatic patient and a normal control, respectively. Biopsy cell samples and biopsy sections were hybridized with an FITC-labeled antisense riboprobe complementary to eotaxin mRNA. The majority of positively hybridized cells in the BAL exhibited a morphology consistent with the macrophages (arrowheads). Fig. 19E shows immunohistochemical detection of eotaxin in a representative biopsy section of an asthmatic patient. Eotaxin immunoreactivity was visualized with the fast red chromogen and localized to the epithelial and inflammatory cells (arrowheads). Fig. 19F is a colocalization of eotaxin immunoreactivity (red) to CD-68 positive macrophages (brown) in BAL cells from an asthmatic patient by double immunohistochemistry. Examples of double positive cells are indicated with arrowheads. Figs. 19G and 19H show eotaxin immunofluorescent staining of BAL cells obtained from an asthmatic patient and a normal control, respectively. Note the eotaxin immunostaining in eosinophils (arrowheads).

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The contribution of the different chemokines present in the lungs of allergic patients with asthma to chemotaxis of purified eosinophils has also been assessed. Accordingly, lung bronchoalveolar lavage was performed in asthmatics. The supernatant was concentrated 10-fold with centricon™ columns. The inhibitory effect of antibodies directed against different chemokines on eosinophil migration in response to BAL fluid is assessed in Table 2. BAL fluid was preincubated with buffer, control Abs, polyclonal rabbit anti-eotaxin, anti-MCP-4, anti-RANTES Abs or a combination of these Abs for one hour before the chemotaxis assay was performed. The concentration of the eotaxin used in the BAL in each assay is indicated. Experiments were performed with a 48-well micro-chemotaxis chamber (NeuroProbe). Migration of human eosinophils was performed on a polycarbonate filter (5 µm pore size). Eosinophils (2 X 10⁶ cells/ml) were resuspended in RPMI medium, loaded into the chambers, incubated at 37°C., 5% CO₂ for 60 min. and the filters were fixed and stained with a RAL kit (Labonord, France). Eosinophils were counted by microscopy in five selected high power fields (magnification x 400). For comparison of results from different chemotaxis assay, a chemotactic index (CI) was calculated as follows: $CI = \frac{\text{Counts-test sample}}{\text{Counts-control medium}}$. In the formula counts-test sample represents the number of migrated cells toward BAL or eotaxin, counts-control is the mean migration of cells in response to RPMI. The percentage of inhibition of locomotion and the confidence interval are presented for experiments performed on eosinophils obtained from 3 individuals. Percentage of inhibition was calculated by the formula: $100 - \{(\text{mean no. of}$

migrated cells in Ab-treated fluids)/(mean number of
migrated cells in untreated fluid)} x 100.

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Table 2
Inhibitory effect of antibodies (Abs) on eosinophil migration in response to bronchoalveolar lavage (BAL) fluid

| BAL Eotaxin Concentration (pg/ml) | BAL/4 | % Inhibition of Migration | | | | | NRS |
|---|-------|---------------------------|--------------------------|------------------------|--|--|------|
| | | Anti-Eotax ^a | Anti-RANTES ^a | Anti-MCP4 ^a | Anti-Eotax + Anti-RANTES ^b | Anti-Eotax + Anti-MCP4 ^b | |
| 714.4 | 0 | 21.40 | 15.23 | 13.33 | 25.70 | 30.48 | 0.95 |
| 504.6 | 0 | 29.03 | 10.96 | 12.26 | 36.13 | 34.19 | 4.5 |
| 453.2 | 0 | 32.90 | 18.30 | 15.24 | 43.90 | 35.97 | 4.9 |

^a p < 0.05 for the confidence interval compared with BAL sample alone; and

^b p < 0.01 for the confidence interval compared with BAL sample alone.

NRS: Normal Rabbit Serum

In Table 2, the 3 chemokines that act mostly through the CCR3 receptor account for approximately 50% of the chemotaxis of eosinophils in asthmatic BAL.

These results show that chemokines (that act
5 through the CCR3 receptor) are increased and important in allergic asthma and inhibition of the CCR3 receptor with antisense oligonucleotides is thus important in the therapy of allergy and asthma.

Experiments were performed to determine whether
10 antisense oligonucleotides directed against the CCR3 receptor could inhibit the mRNA expression of this receptor. It is to be noted in Fig. 20 that the antisense oligonucleotide directed against the CCR3 receptor (CCR3AS 86 (RC86A): 5'-CTGGGCCATC AGTGCTCTG-3'
15 SEQ ID NO:18) is effective at inhibiting the expression of mRNA for CCR3 in HL60 cells that have been differentiated into the eosinophil pathway. The housekeeping gene that was used to standardize the mRNA preparation was G3PDH (451bp band, gel on the right).
20 The gel on the left shows that when compared to the sense oligonucleotide (CCR3SS 86: 5'-CAGAGCACTG ATGCCCCAG-3'; SEQ ID NO:19), RC86A inhibits mRNA for CCR3 (middle lane). HL60 cells (clone 15) were differentiated into eosinophil like cells by incubation
25 for 10 days in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin, streptomycin, l-glutamine and 0.5 molar acetic acid at 37°C in 5% CO₂ with medium alone, 10µM CCCR3AS1 or 10 µM CCR3Ss for 6 hours. mRNA was isolated with trizol and after
30 standardization of the amount of mRNA with G3PDH, the expression of mRNA for CCR3 was assessed in mRNA obtained from cells cultured in medium (control), with CCR3AS 86 (middle lane) or with CCR3SS 86 (right lane).

Experiments were also performed to assess
35 whether antisense oligonucleotides directed against the

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CCR3 receptor could inhibit the mRNA expression of this receptor on Ghost cells transfected with the CCR3 receptor. These cells were obtained from the NIH and the CCR3 gene was introduced via retroviral infection with MLV BABE-puro vector. It is to be noted in Fig. 21, gel on the right, that the antisense oligonucleotide directed against the CCR3 receptor (RC86A) is effective at inhibiting mRNA expression of the CCR3 receptor when compared to control not incubated with 10 μ Mol antisense. The gel on the left shows the results obtained for the housekeeping gene G3PDH.

Additional experiments were performed to assess whether antisense oligonucleotides directed against the CCR3 receptor could inhibit the function of this receptor. It is to be noted in Fig. 22 that the antisense oligonucleotide directed against the CCR3 receptor (RC269AS: 5'-CCCTGACATA GTGGATC-3' SEQ ID NO:20) and RC86A are effective at inhibiting calcium mobilization (a sign of chemotaxis) in eosinophils. Human eosinophils were purified from blood obtained from patients with asthma by Ficoll Hypaque centrifugation, red blood cell lysis, followed by negative selection with anti-CD16 coated magnetic beads on a MACS cell sorter (to eliminate neutrophils). The eosinophils were then washed and incubated with recombinant human IL-5 (1.6 ng/ml) in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin, streptomycin, and l-glutamine at 37°C in 5% CO₂ overnight. The cells were washed and then incubated in RPMI 1640 during 4 hours in the presence or absence of 10 μ M RC269AS or 10 μ M RC269S 5'-GATCCACTAT GTCAGGG-3' (SEQ ID NO:21) washed and resuspended at 2.5x10⁶ cells/ml in RPMI 1640 and incubated with Fura-2M at a concentration of 3 μ M for 45

minutes. The cells were washed twice with PBS and resuspended in calcium buffer (PBS, 1mM HEPES, 1mM Ca⁺⁺). Calcium efflux after immediate stimulation with eotaxin was measured with a Perkin Elmer L50B Spectrophotometer (excitation at 340nm, slit 5 nm and emission at 492nm, slit 5nm).

Experiments were then performed to determine whether antisense oligonucleotides directed against the CCR3 receptor could inhibit the influx of eosinophils *in vivo*. It is to be noted in Fig. 23 that the phosphorothioate antisense oligonucleotide RCC3A4 5'-ACTCATATTC ATAGGGTG-3' (SEQ ID NO:22) is effective at inhibiting mRNA expression of rat CCR3 *in vitro*. On the left are results of semi-quantitative RT-PCR for the G3PDH gene in control medium without antisense, in medium with CCR3A2 (5'-GCCAACACAG CATGAACG-3', SEQ ID NO:23) an antisense that has proven to be ineffective against CCR3 receptor expression and in medium with RCC3A4 (10 μ Mol) treated marrow cells. On the right, from right to left are the results of semiquantitative RT-PCR of the rat CCR3 receptor; lane 1 shows the inhibition of gene expression with RCC3A4. Brown Norway rats were anesthetized with pentothal (65 mg/Kg i.p.). They were then killed, lower extremities retrieved and femurs isolated. The bones were incubated in 70% ethanol for 10 minutes. The two extremities of the bones were sectioned and bone marrow obtained by injection and aspiration of 15 ml of RPMI 1640. The cells were washed twice and incubated for 6 hours in RPMI 1640 at 37°C in 5% CO₂ with medium alone, medium with 10 μ M of RC3A2 or with 10 μ M RCC3A4.

Additional experiments show that antisense oligonucleotides directed against the CCR3 receptor are effective at inhibiting eosinophil influx into the peritoneal cavity and into the lungs *in vivo*. Brown

Norway rats were actively sensitized to ovalbumin by injecting 1.25 mg ovalbumin mixed with 200 mg aluminum hydroxide sub-cutaneously. On day 14, rats received 500 micrograms of RCC3A4 in 500 μ l of 0.9%NaCl or 0.9%NaCl intra-peritoneally. The next day the rats were challenged by administering 1.25mg of ovalbumin intra-peritoneally. Eight hours later the rats are anesthetized with 65 mg/Kg of pentothal, the abdominal cavity opened and washed with 8 ml of RPMI 1640. The washing solution is retrieved, cells are washed, counted and centrifuged onto slides in a Cytospin III. A differential cell count is performed. It is to be noted in Fig. 18A that the antisense oligonucleotide RCC3A4 directed against the CCR3 receptor inhibits eosinophil influx into the peritoneal cavity by approximately 60%.

In additional experiments Brown Norway rats were actively sensitized to ovalbumin by injecting 1.25 mg ovalbumin mixed with 200 mg aluminum hydroxide sub-cutaneously. On day 14, rats received 500 μ g of RCC3A4 in 500 μ l of 0.9%NaCl or 0.9%NaCl intra-peritoneally. The next day, after general anesthesia with 65 mg/Kg pentothal and endotracheal intubation, 200 μ g of the antisense is administered intra-tracheally in 50 μ l of 0.9%NaCl. Twenty minutes later ovalbumin challenge is performed by injecting 200 micrograms of ovalbumin in 50 μ l intra-tracheally. After 8 hours, the rats are again intubated after general anesthesia and a lung lavage consisting of 5 times 5 ml instillations is performed. Cells are washed, counted and centrifuged onto slides in a Cytospin III. A differential cell count is performed. It is to be noted in Fig. 18B hat the antisense oligonucleotide RCC3A4 directed against the CCR3 receptor inhibits eosinophil influx into the lungs by approximately 66%.

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These results show that antisense oligonucleotides directed against the CCR3 receptor are important in the prevention of the eosinophil influx that occurs in different allergic diseases and thus their effects.

Furthermore, priming with the cytokine IL-5 (which acts through the IL-5 receptor) can either increase the chemotaxis of cells or the release of chemokines when the cells are stimulated.

Fig. 24 shows that priming of eosinophils with IL-5 increases the chemotaxis of eosinophils when they are stimulated with eotaxin. Preincubation of eosinophils with IL-5 (which acts through the IL-5 receptor) increases the chemotaxis induced by eotaxin at every dose tested. The peak of chemotaxis is higher with priming which suggests a synergistic effect of IL-5 on the effects of eotaxin. Dose-response curves show the chemotactic activity of purified human eosinophils to eotaxin (filled squares) and transmigration through a polycarbonate filter after preincubation with IL-5 (closed circles). Mononuclear cells and granulocytes were purified from peripheral blood by Ficoll-Paque (Pharmacia) density centrifugation. Granulocytes were obtained by dextran sedimentation. Human eosinophils were further purified by negative selection with anti-CD16 and anti-CD3-coated immunomagnetic microbeads using a Magnetic Cell Sorting System (Miltenyi Biotec) at 4°C. The degree of purity of eosinophil populations, estimated after staining with Giemsa, was between 92 and 100%. Results are presented as mean \pm SD of 5 high power fields. Control serum had no effect on chemotaxis. Results identified by "*" represent a probability of being different of $p < 0.01$ compared with unprimed eosinophils at each concentration of eotaxin.

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Figs. 25a to 25I show that priming of eosinophils with IL-5 increases the amount of chemokines in the cells and increases their release after stimulation with immunoglobulin. Preincubation of eosinophils with IL-5 overnight increased the expression of eotaxin (Fig. 25A) and MCP-4 (Fig. 25B) when compared to controls (Fig. 25F). When eosinophils are stimulated with IgE-anti-IgE they will also release eotaxin (Fig. 25C, 25D, 25E) or MCP-4 (Fig. 25G, 25H, 25I). Eosinophils were purified as described above and incubated overnight with recombinant human IL-5 (1 ng/ml). This incubation increased eotaxin (Fig. 25A) and MCP-4 (Fig. 25B) in cells when compared to control cells incubated in medium alone (Fig. 25F). Stimulation of the eosinophils by a preincubation with IgE for 15 minutes then exposure to anti-IgE lead to a progressive release eotaxin (Figs. 25C, 25D, 25E) or MCP-4 (Figs. 25G, 25H, 25I) at 15 minutes (Figs. 25C, 25G), 2 hours (Figs. 25D, 25H) or 12 hours (Figs. 25E, 25I).

Accordingly, the combination of antisense oligonucleotides in accordance with the present invention, that are directed against different receptors (for example the IL-5 and the CCR3 receptors) have a synergistic effect in the therapy of allergy, asthma or neoplastic cell proliferation.

Experiments were performed in Brown Norway rats in order to assess whether the combination of the antisense phosphorothioate RB141A directed against the common beta chain of the GM-CSF, IL-3 and IL-5 receptors and the antisense phosphorothioate RCC3A4 directed against the CCR3 receptor had synergistic effects on eosinophil recruitment into the lungs after antigen challenge when compared to either one alone. Brown Norway rats were actively sensitized to ovalbumin

by injecting 1.25 mg ovalbumin mixed with 200 mg aluminum hydroxide sub-cutaneously. On day 14, rats received 500 μ g of RCC3A4 and 500 μ g of RB141A in 500 μ l of 0.9%NaCl or 0.9%NaCl intra-peritoneally. The next
5 day, after general anesthesia with 65 mg/Kg pentothal and endotracheal intubation, 180 μ g of each antisense is administered intra-tracheally in 60 μ l of 0.9%NaCl. Twenty minutes later ovalbumin challenge is performed by injecting 200 micrograms of ovalbumin in 60 μ l
10 intra-tracheally. After 8 hours, the rats are again intubated after general anesthesia and a lung lavage consisting of 5 times 5 ml instillations is performed. Cells are washed, counted and centrifuged onto slides in a Cytospin III. A differential cell count is
15 performed. It is to be noted in Fig. 18B that the combination of antisense oligonucleotide RCC3A4 and RB141A directed against the CCR3 receptor and common beta sub-unit of the GM-CSF, IL-3 and IL-5 receptors have a synergistic effect on eosinophil influx into the
20 lungs. The inhibition of eosinophil influx was approximately 90%.

The antisense oligonucleotides of the present invention when compared to the use of soluble IL-4 receptors in allergy and asthma has the following
25 advantages: a) as shown in example 1, the much smaller size of these molecules permits them to diffuse into the tissues and penetrate the cells that are expressing the receptors (epithelial cells, smooth muscle cells); b) the use of an antisense oligonucleotide against the
30 common sub-unit of the IL-4 and IL-13 receptor permits a broader effect by blocking the effects of IL-13 that are similar to those of IL-4 in many respects on IgE production, as IL-13 is also increased in allergy and asthma; and c) the combination of anti-receptor
35 oligonucleotides against receptors for many cytokines

(IL-3, IL-5 and GM-CSF or IL-4 and IL-13 or CCR3 (eotaxin, RANTES and MCP-4)) will permit broader effects in a disease where a certain individual's heterogeneity in the inflammatory cascade exist.

5 Furthermore, the antisense oligonucleotides of the present invention have the following advantages: a) the antisense anti-receptor oligonucleotides will act directly on tissue or inflammatory cells that are present at the site of administration and not
10 indirectly by potentially blocking the release of mediators (if directed against the cytokines themselves); b) the antisense anti-receptor oligonucleotides will not be affected by diffusion of cytokines that are produced and increased in the blood
15 of patients with allergy and asthma; and c) one antisense anti-receptor oligonucleotide of the present invention blocks the effects of 2 or 3 mediators which have been shown to be increased in allergy or asthma, thus having a broader effect than one antisense
20 oligonucleotide directed only against one mediator or receptor and therefore being an advantage.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications
25 and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the
30 art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

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CLAIMS

1. An antisense oligonucleotide for treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, said oligonucleotide being directed against a nucleic acid sequence coding for a common subunit of the IL-3, IL-5 and GM-CSF receptors.

2. The oligonucleotide of claim 1, wherein the nucleic acid sequence coding for the receptor is a nucleic acid coding for the common beta sub-unit of the IL-3, IL-5 and GM-CSF receptors.

3 The oligonucleotide of claim 1, wherein said oligonucleotide has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, , SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22 and SEQ ID NO:23.

4. A pharmaceutical composition for treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, said composition comprising at least one antisense oligonucleotide as defined in claim 1, 2 or 3, in association with a pharmaceutically acceptable carrier.

5. Use of an oligonucleotide as defined in claim 1, 2 or 3 for treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer.

6. Use of a pharmaceutical composition as defined in claim 4 for treating and/or preventing asthma,

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allergy, hypereosinophilia, general inflammation or cancer.

7. A method for treating and/or preventing asthma, allergy, general inflammation or cancer, said method comprising the step of administering an effective amount of an oligonucleotide as defined in claim 1, 2 or 3, to a patient in need of such a treatment.

8. Use of an oligonucleotide as defined in claim 1, 2 or 3 for the manufacture of a medicament for treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer.

9. Use of a pharmaceutical composition as defined in claim 4 for the manufacture of a medicament for treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer.

10. A pharmaceutical composition comprising at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors and at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors.

11. The pharmaceutical composition according to claim 10, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

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12. The pharmaceutical composition according to claim 10, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

13. The pharmaceutical composition according to claim 10, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16 and at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

14. The pharmaceutical composition according to claim 10, further comprising at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

15. The pharmaceutical composition according to claim 14, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

16. A pharmaceutical composition comprising at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

17. The pharmaceutical composition according to claim 16, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

18. The pharmaceutical composition according to claim 16, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

19. The pharmaceutical composition according to claim 16, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16 and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

20. A pharmaceutical composition comprising at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

21. The pharmaceutical composition according to claim 20, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

22. The pharmaceutical composition according to claim 20, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

23. The pharmaceutical composition according to claim 20, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

24. A method of treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, the method comprising administering to a patient at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors and at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors.

25. The method according to claim 24, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

26. The method according to claim 24, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

27. The method according to claim 24, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16; and at least one oligonucleotide directed

against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

28. A method of treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, the method comprising administering to a patient at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors, at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors, and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

29. The method according to claim 28, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

30. The method according to claim 28, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

31. The method according to claim 28, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

32. The method according to claim 28, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16; at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, and SEQ ID NO:7; and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO: 20, SEQ ID NO:22, and SEQ ID NO:23.

33. A method of treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, the method comprising administering to a patient at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

34. The method according to claim 33, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

35. The method according to claim 33, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

36. The method according to claim 33, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-3, IL-5 and GM-CSF receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16; and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

37. A method of treating and/or preventing asthma, allergy, hypereosinophilia, general inflammation or cancer, the method comprising administering to a patient at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and

IL-13 receptors and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor.

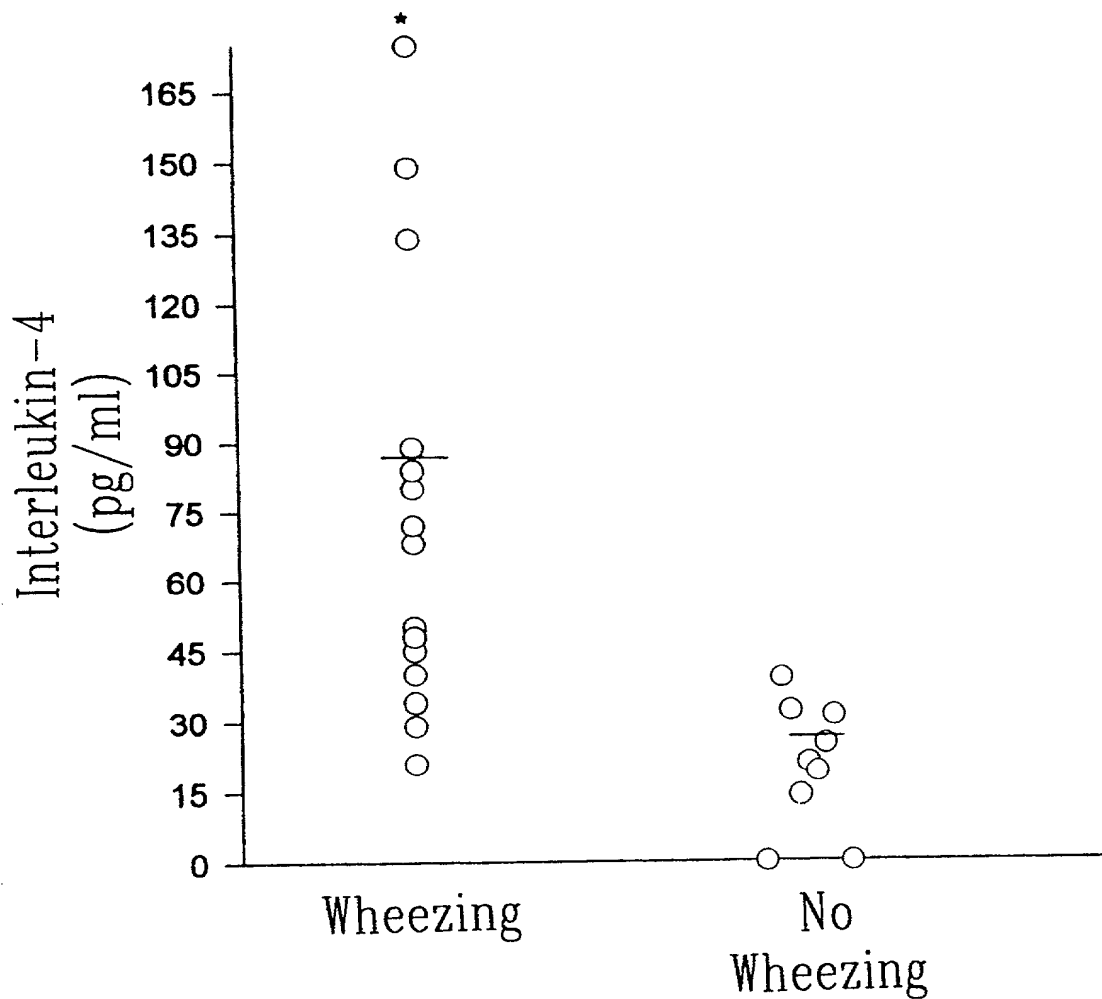
38. The method according to claim 37, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

39. The method according to claim 37, wherein at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

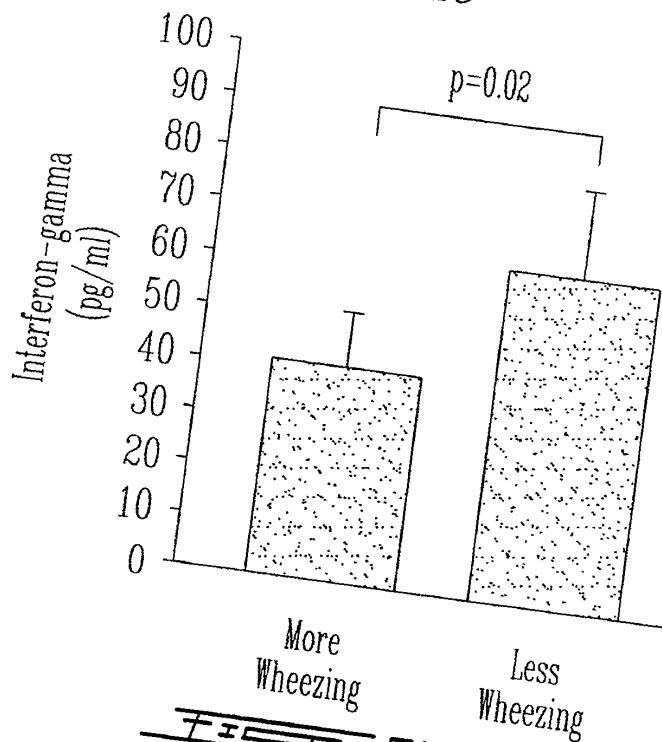
40. The method according to claim 37, wherein at least one oligonucleotide directed against a nucleic acid encoding a common subunit of the IL-4 and IL-13 receptors is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7; and at least one oligonucleotide directed against a nucleic acid encoding a CCR3 receptor is selected from the group consisting of the oligonucleotides listed in the sequence listings as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:23.

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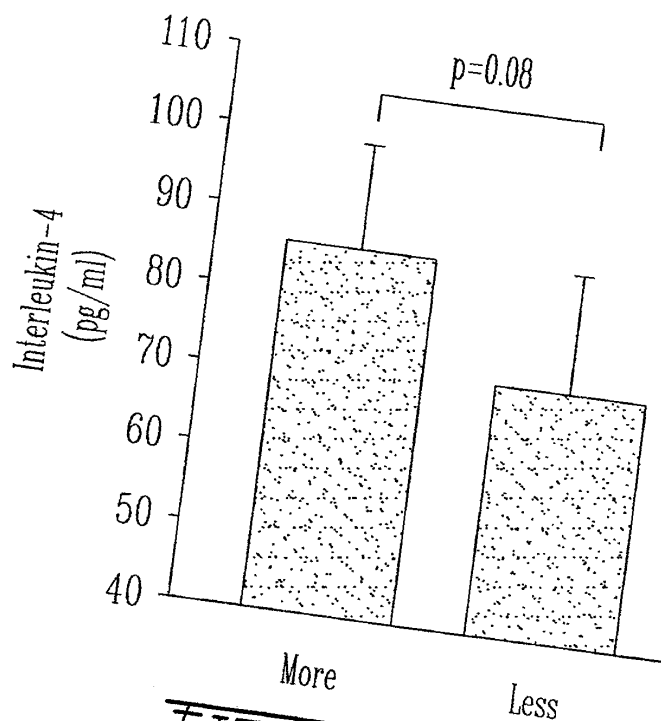
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FIG. 1

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~~FIG. 2A~~



~~FIG. 2B~~

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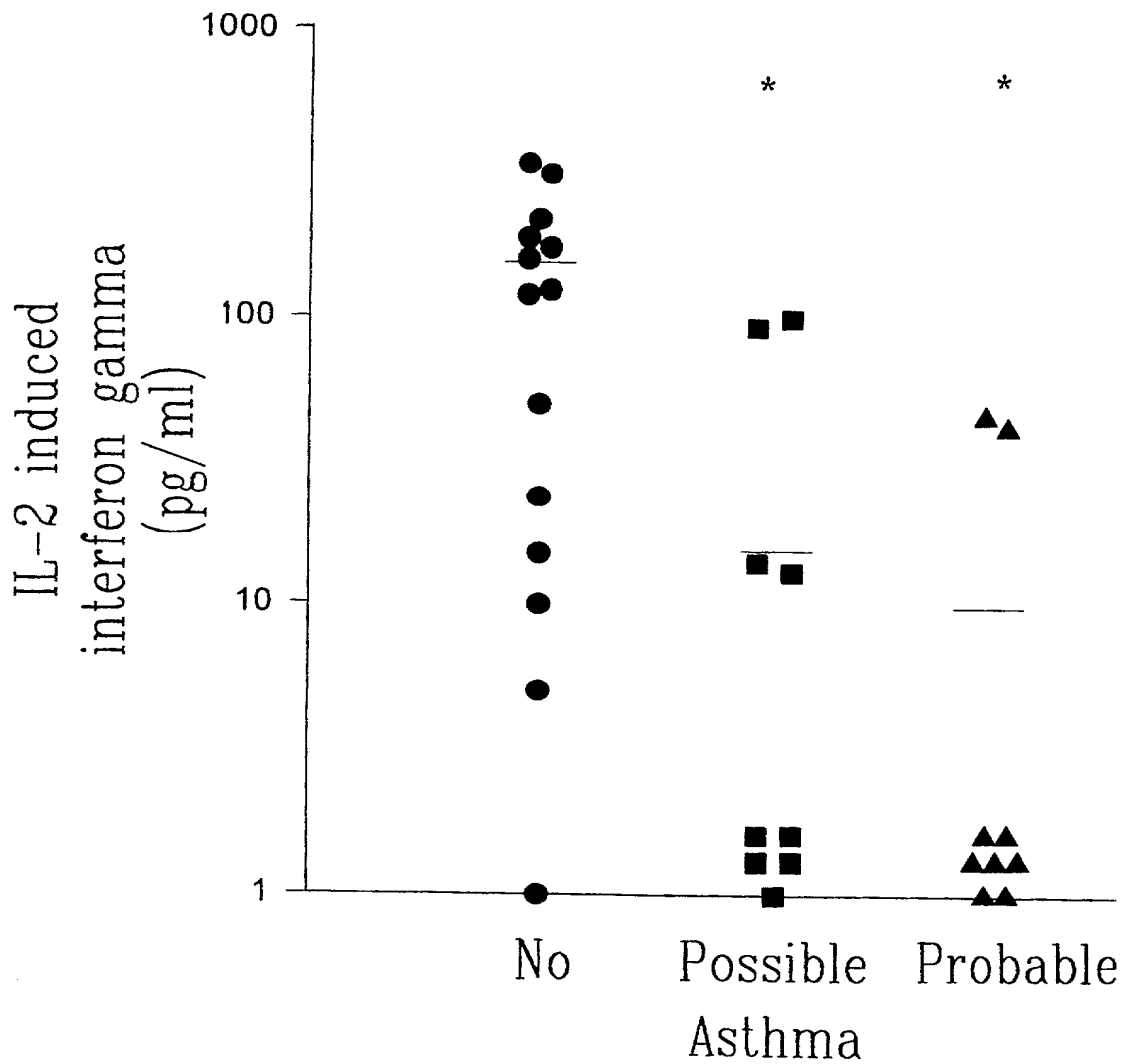


FIG. 3A

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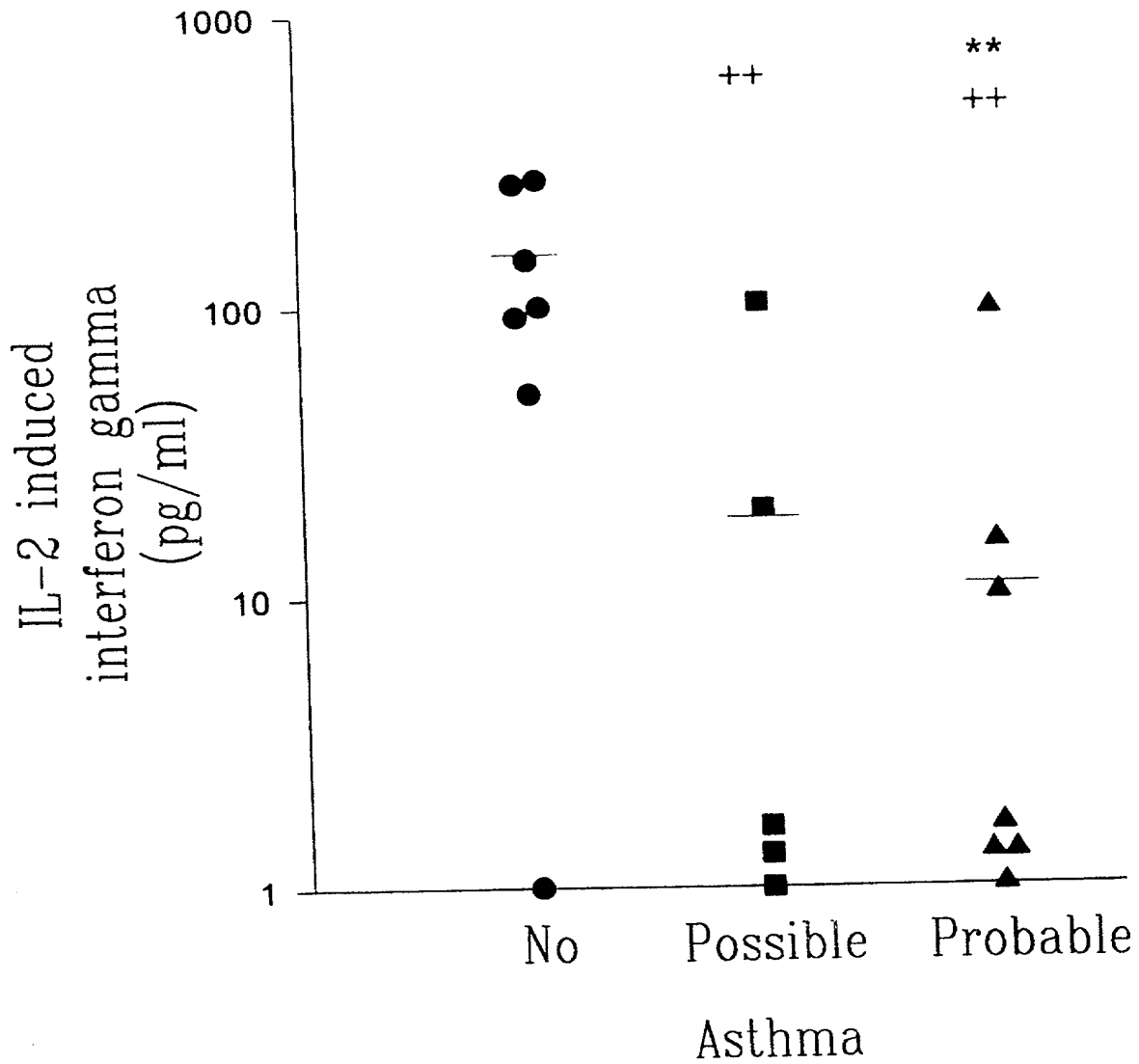


FIG. 3B

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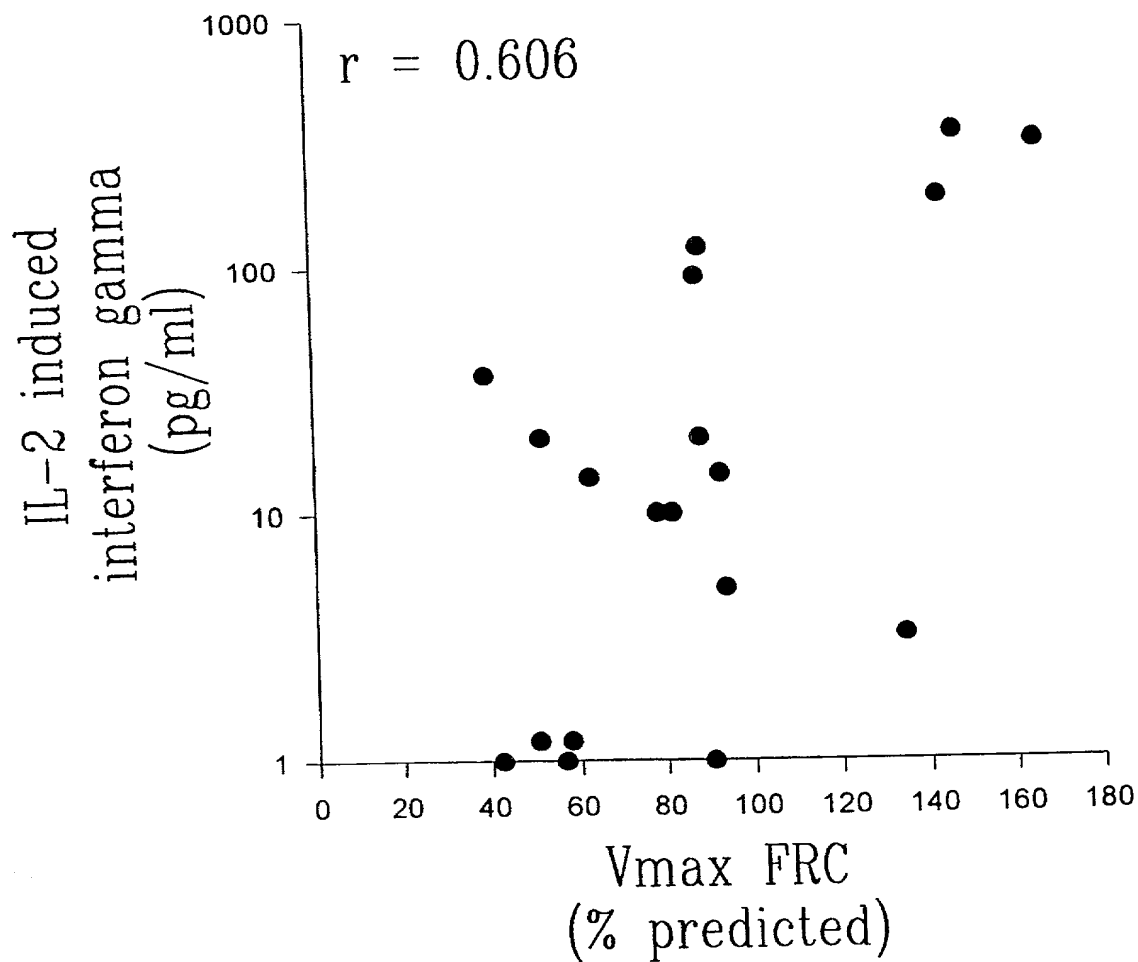


FIG. 4A

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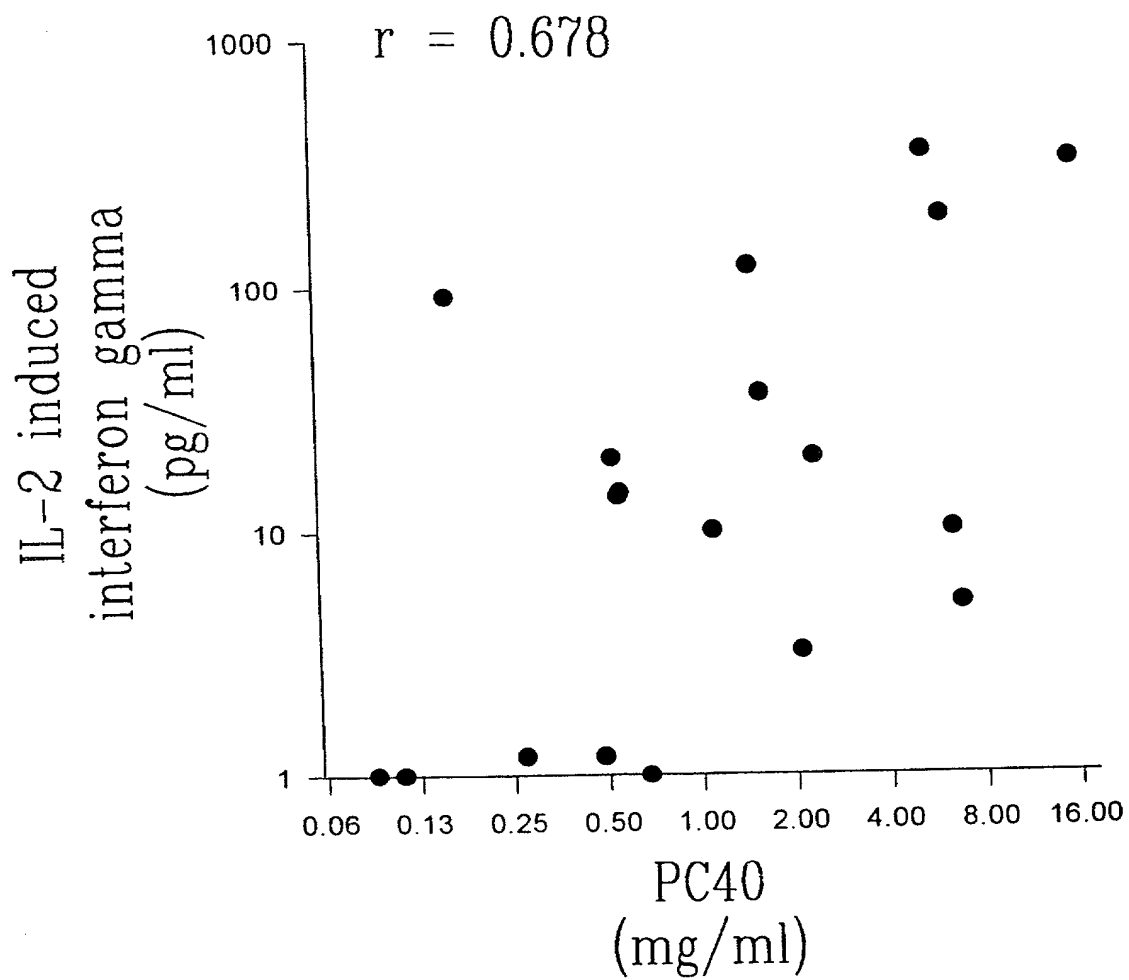


FIG - 4B

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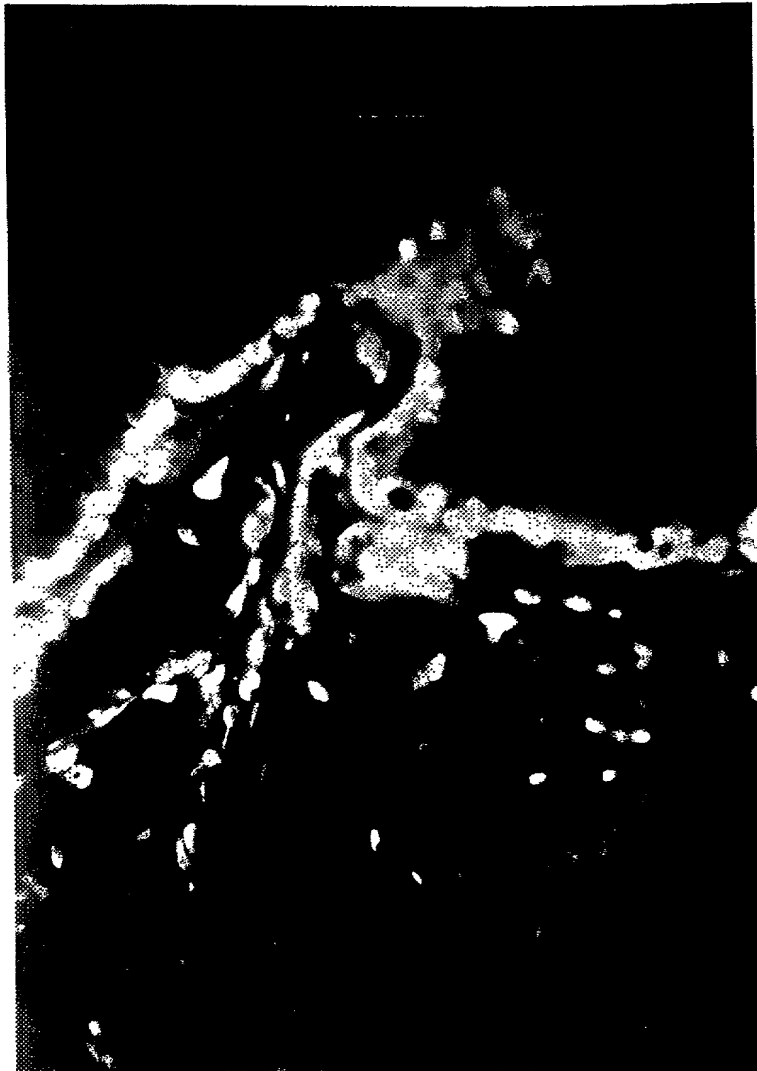


FIG. 5A

SUBSTITUTE SHEET (RULE 26)

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FIG. 5B

SUBSTITUTE SHEET (RULE 26)

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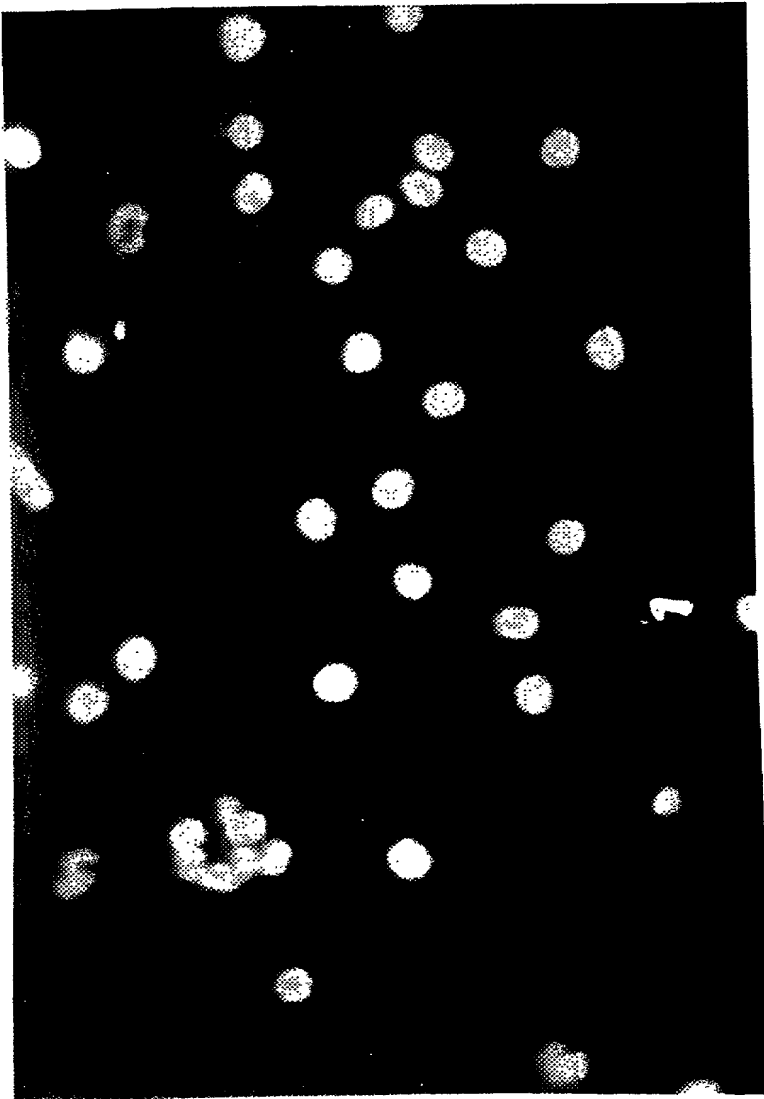


FIG. 1A

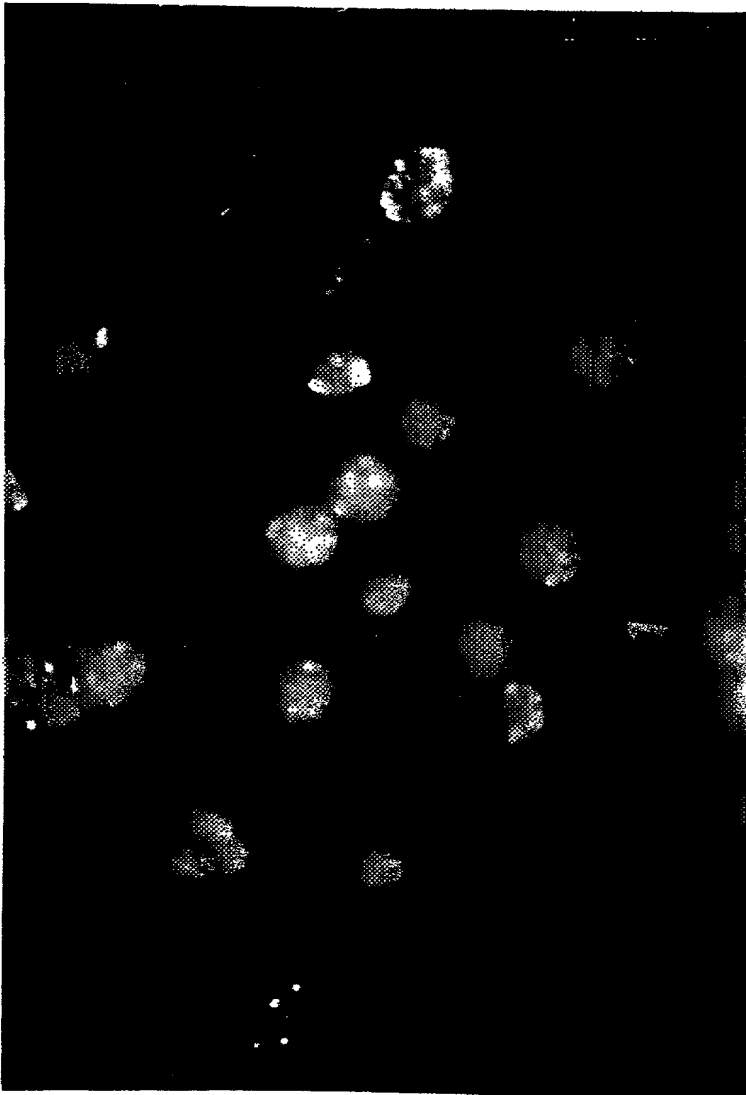
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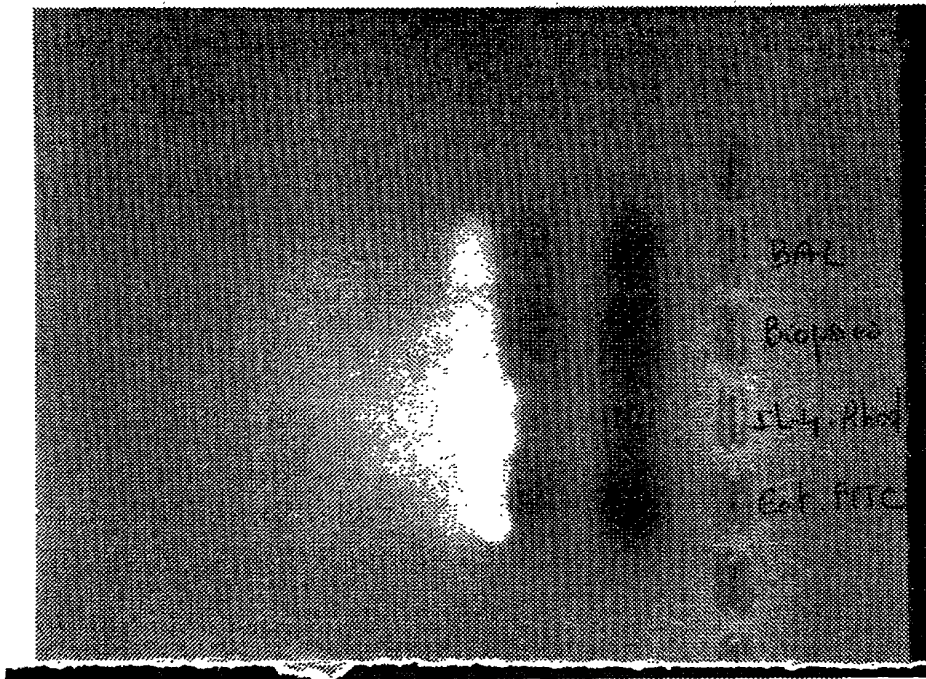


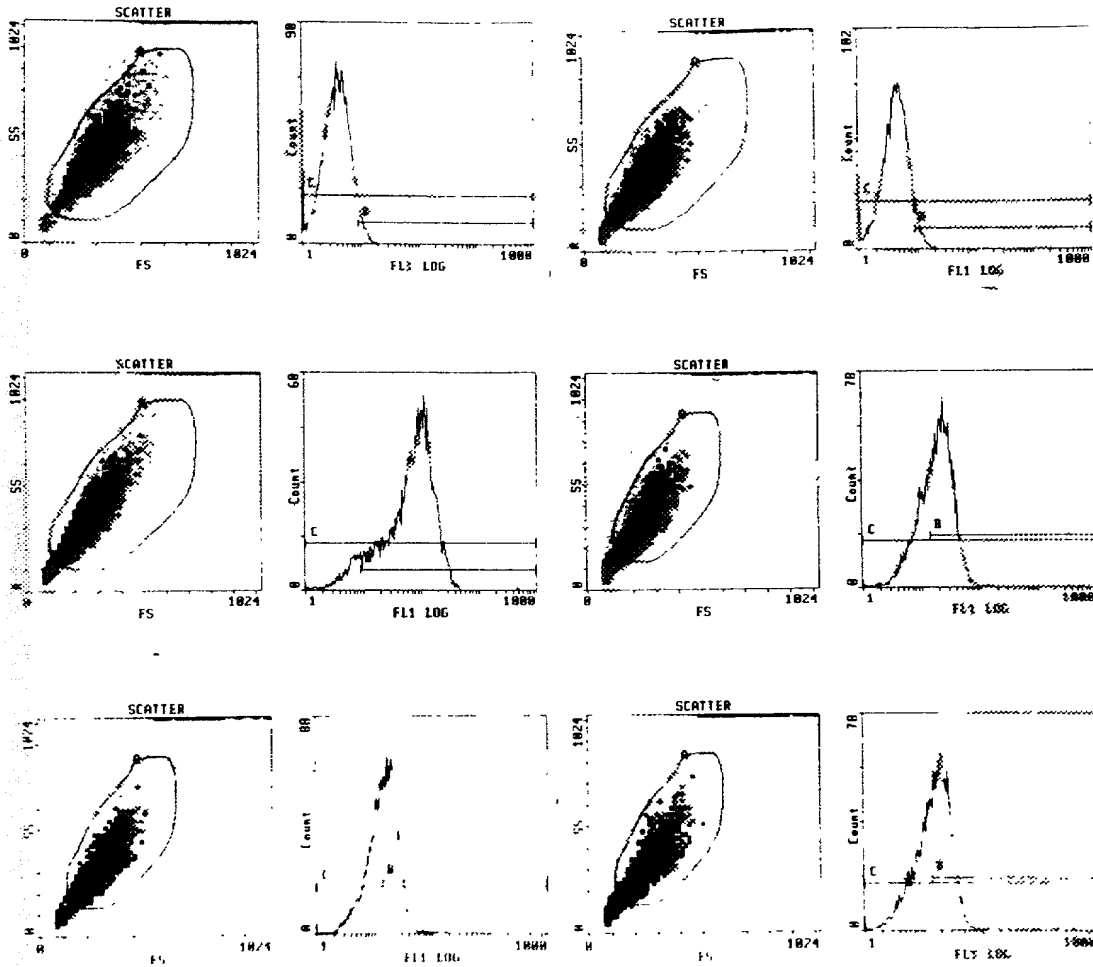
FIG. 7

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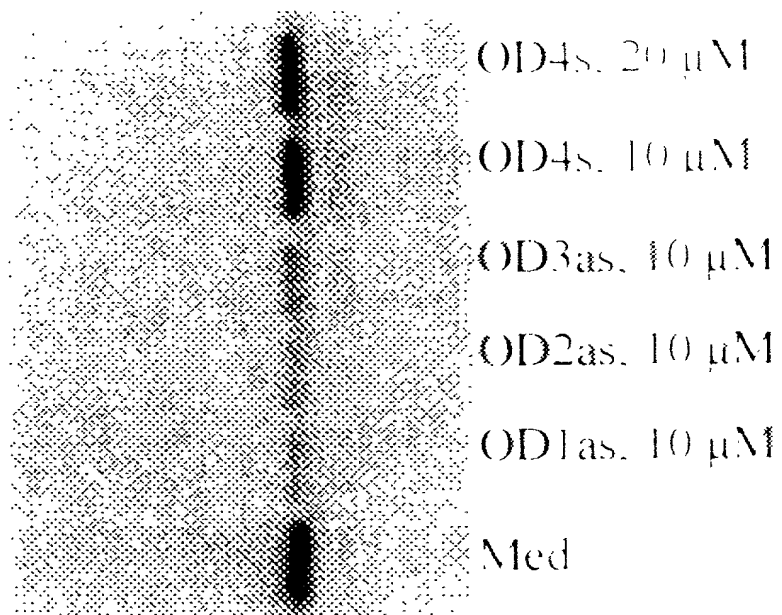
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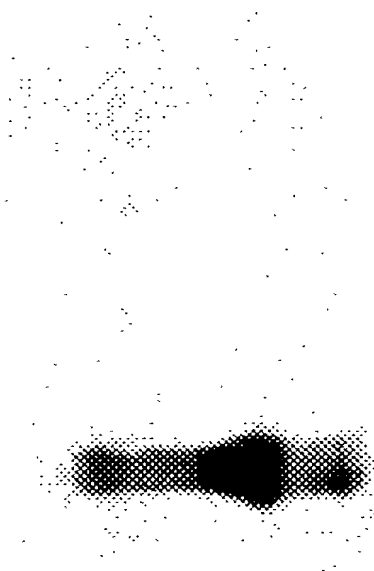


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FIS-9A

↑
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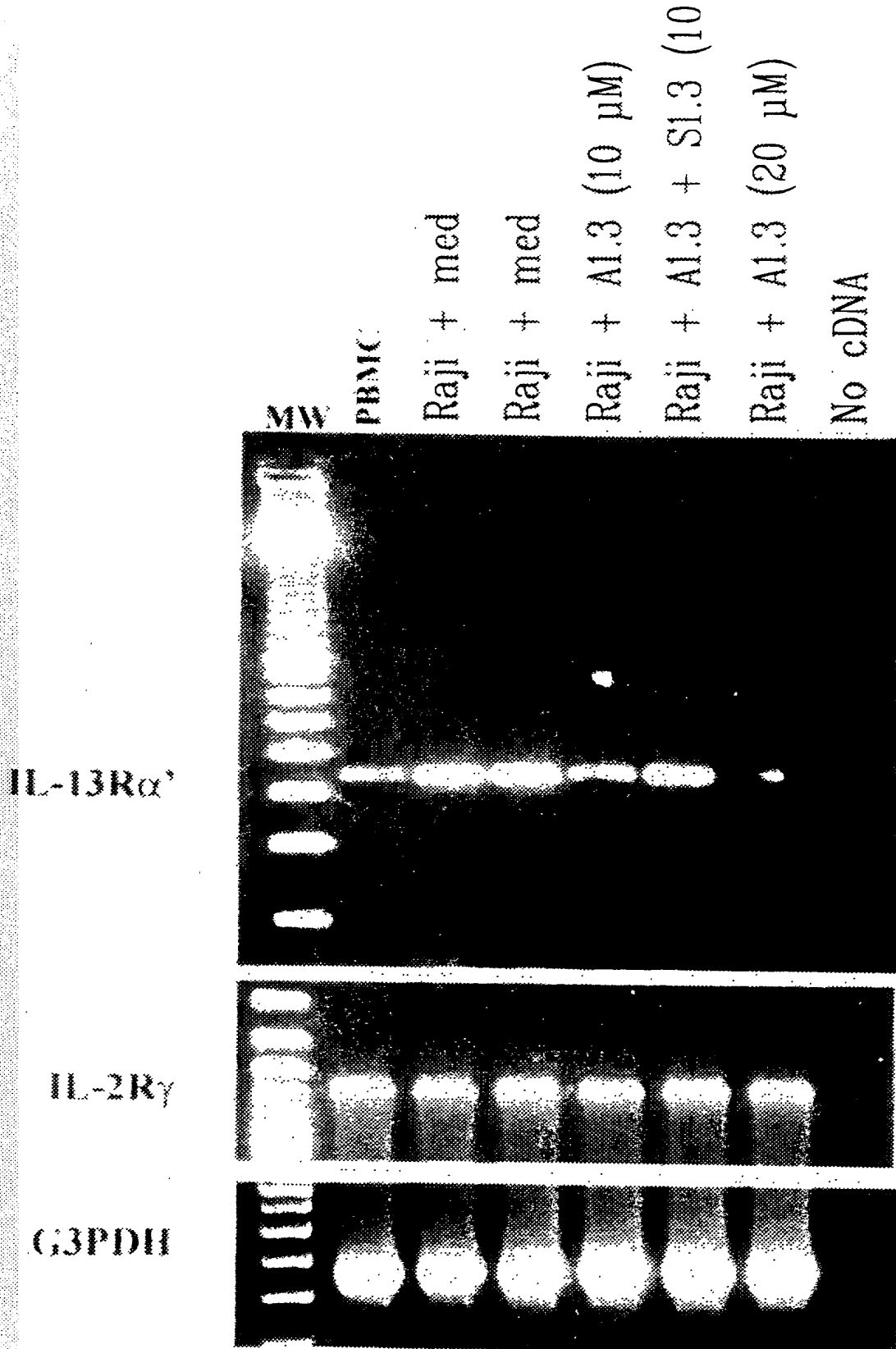
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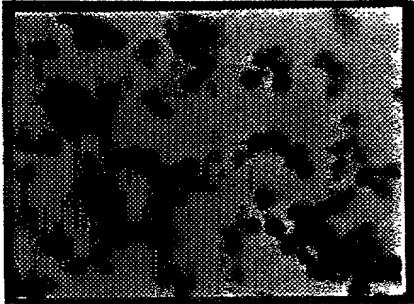
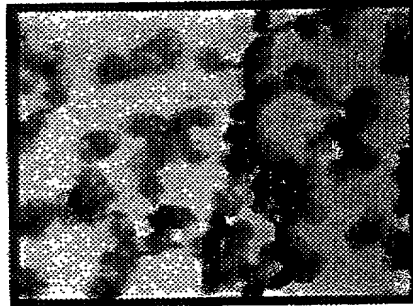
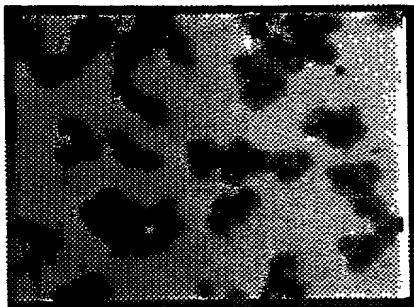
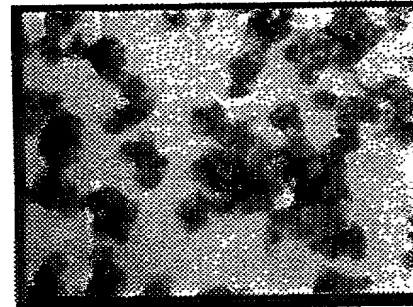
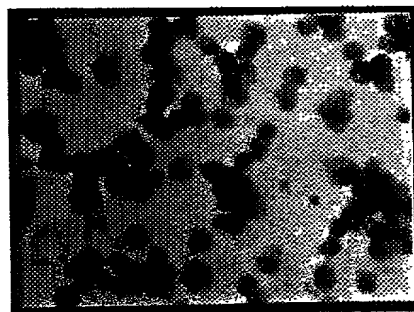
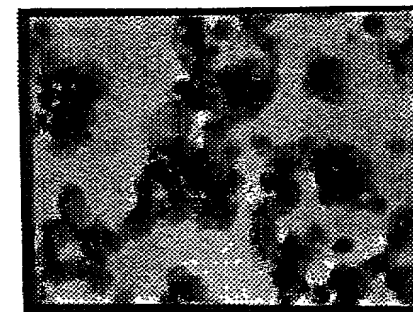
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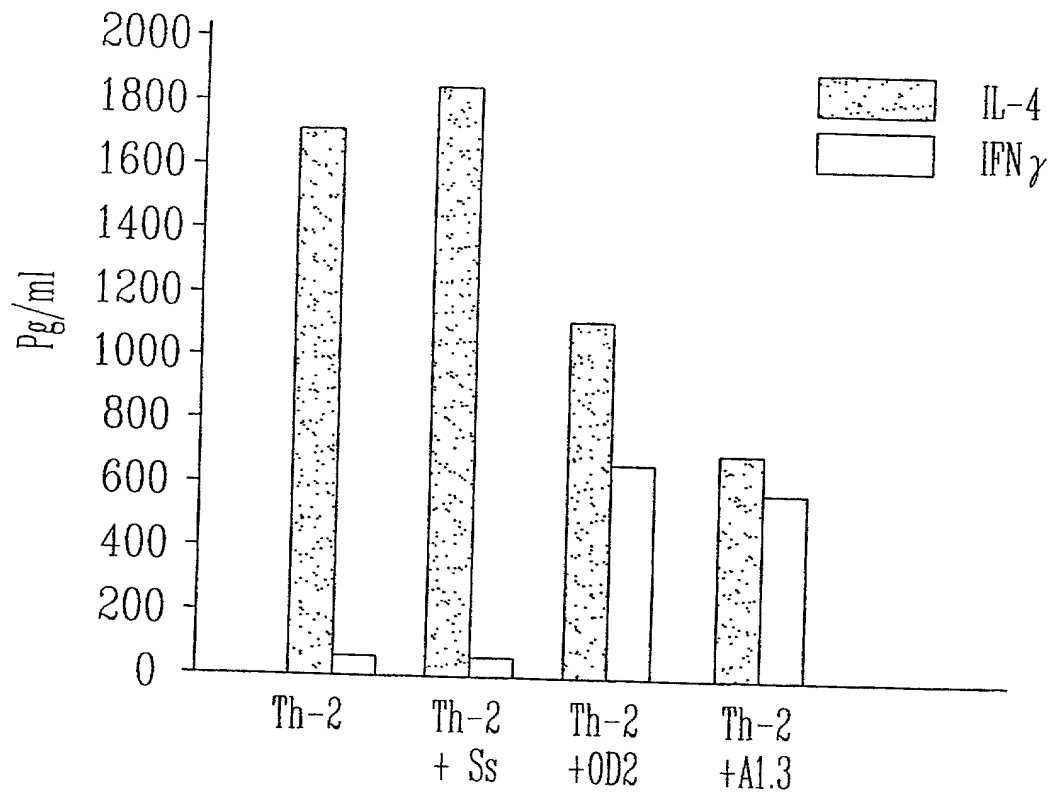
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FIG. 11AFIG. 11DFIG. 11BFIG. 11EFIG. 11CFIG. 11F

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FIG. 12

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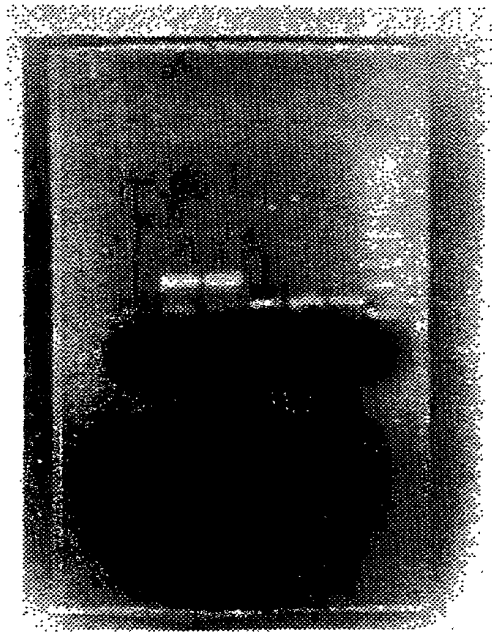
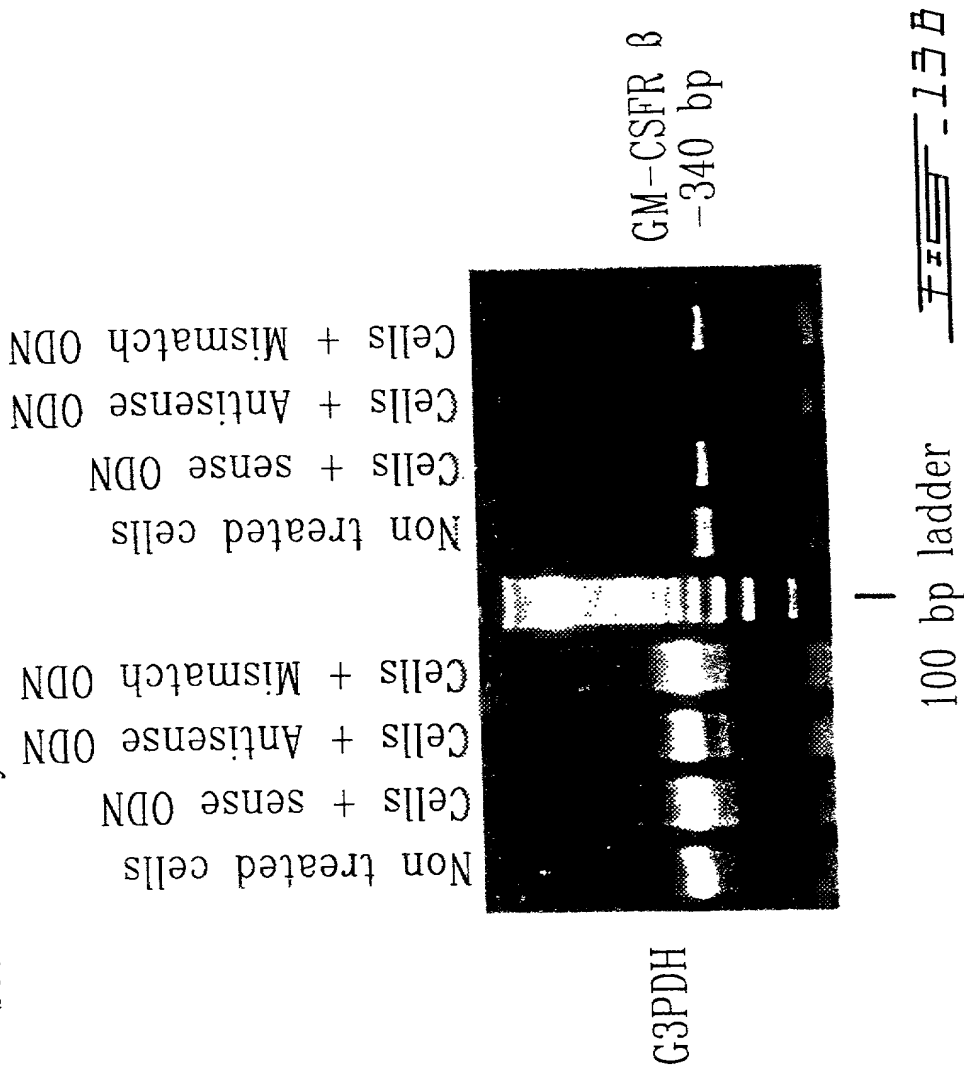


FIG. 13A

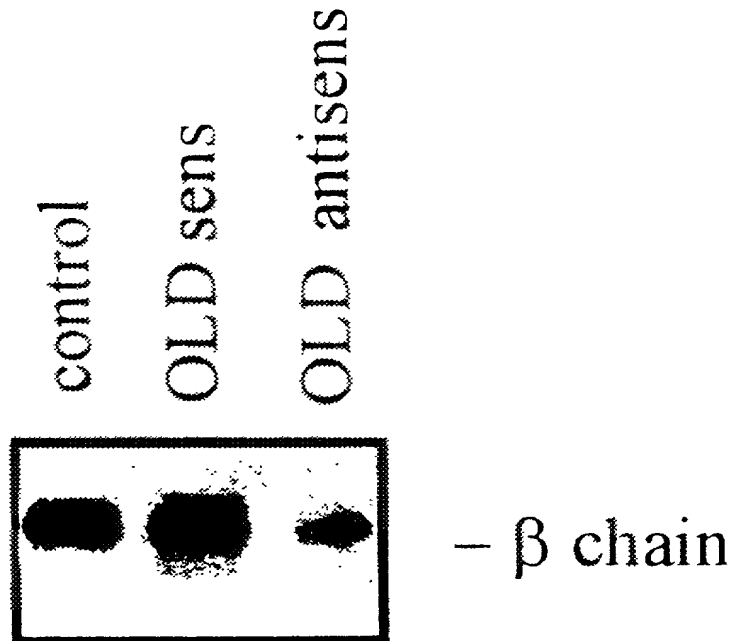
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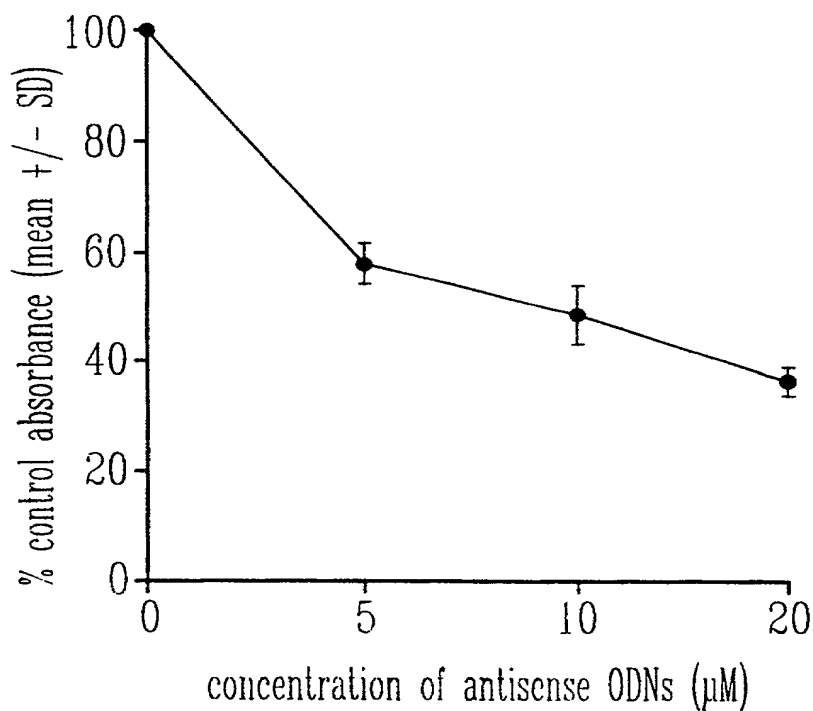
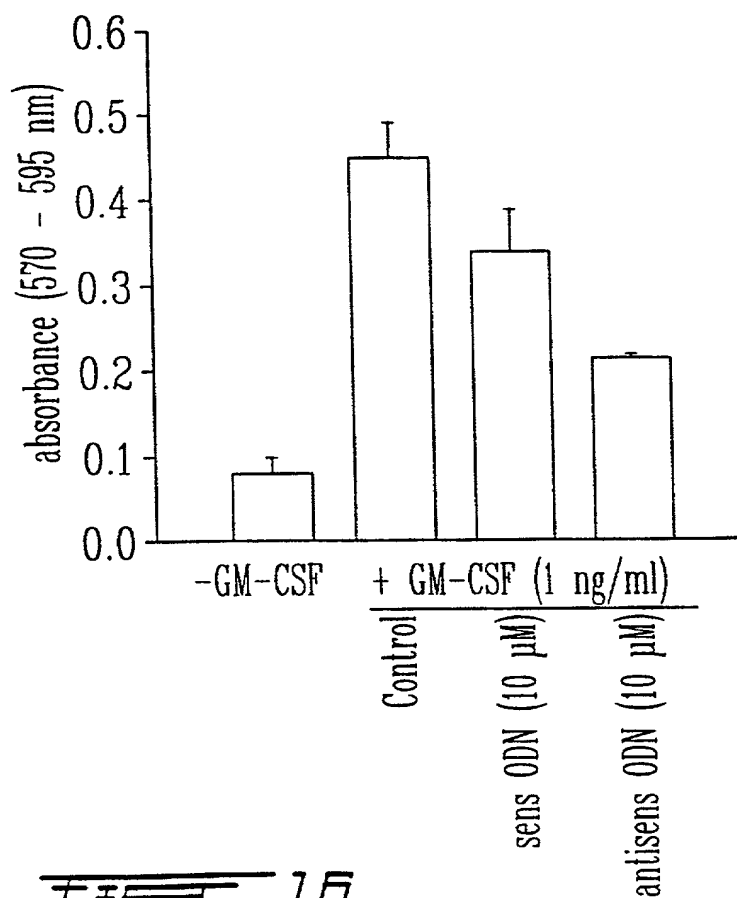
Modulation of expression of mRNA coding for GM-CSFR beta chain by antisense of ODN in U937 cell line.



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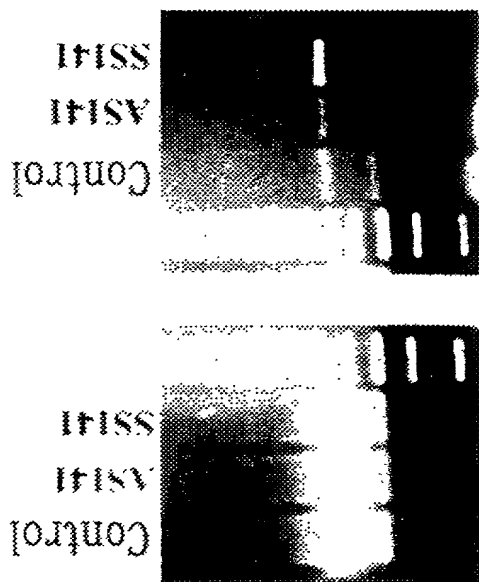
Fig. 14

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FIG. 15FIG. 16

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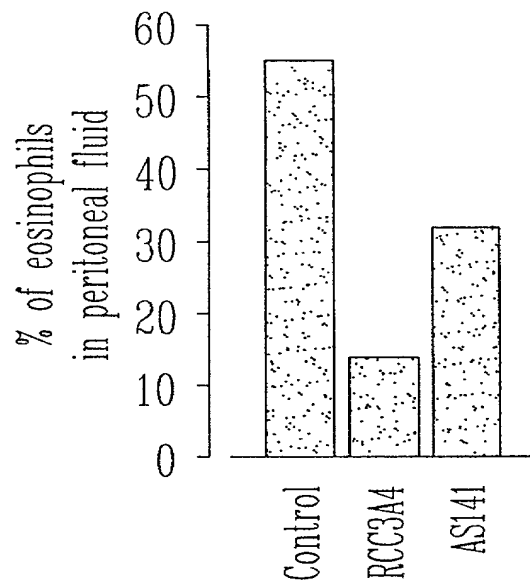
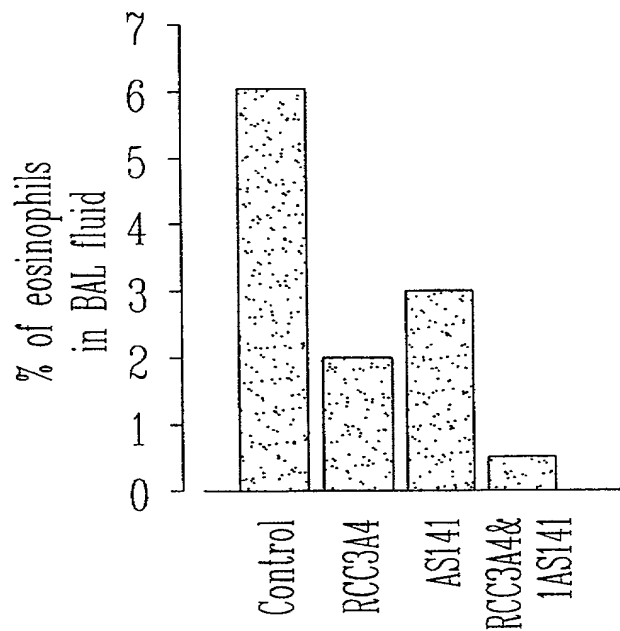
GM-CSFR β



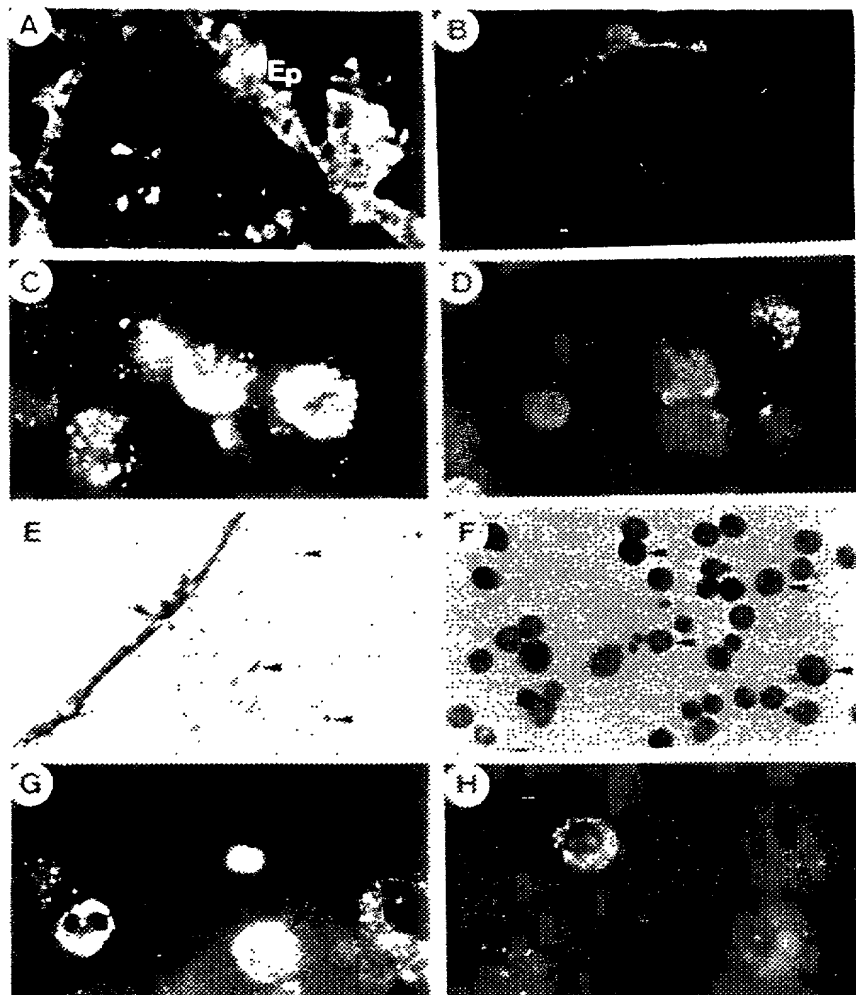
G3PDH

Fig. 17

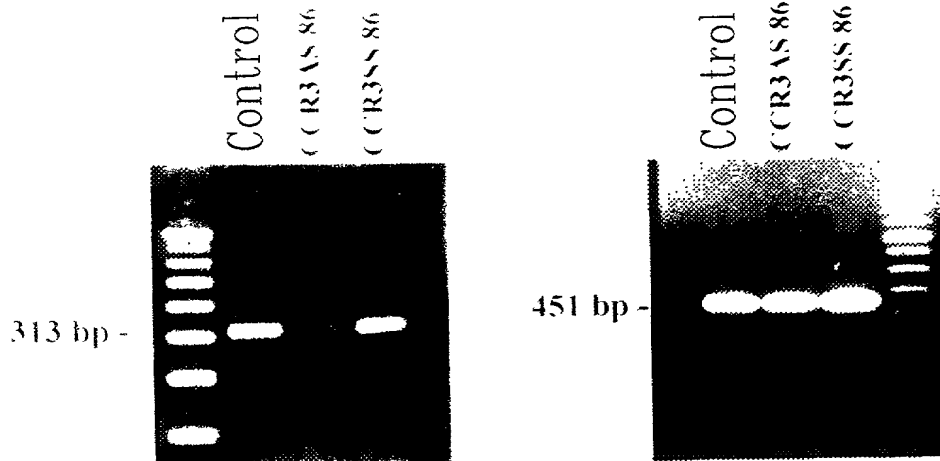
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FIG. 1AFIG. 1B

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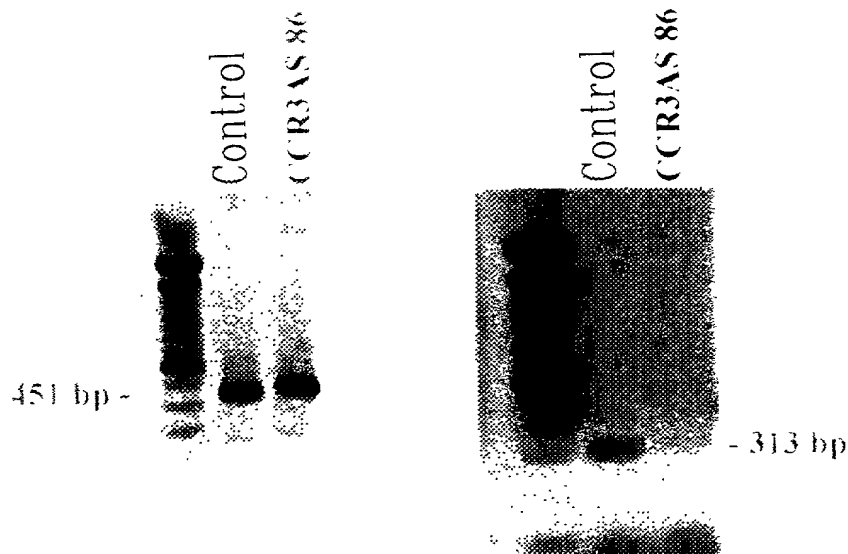
FIG. 19

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CCR3

G3PDH



G3PDH

CCR3

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Eotaxin 40 ng/ml

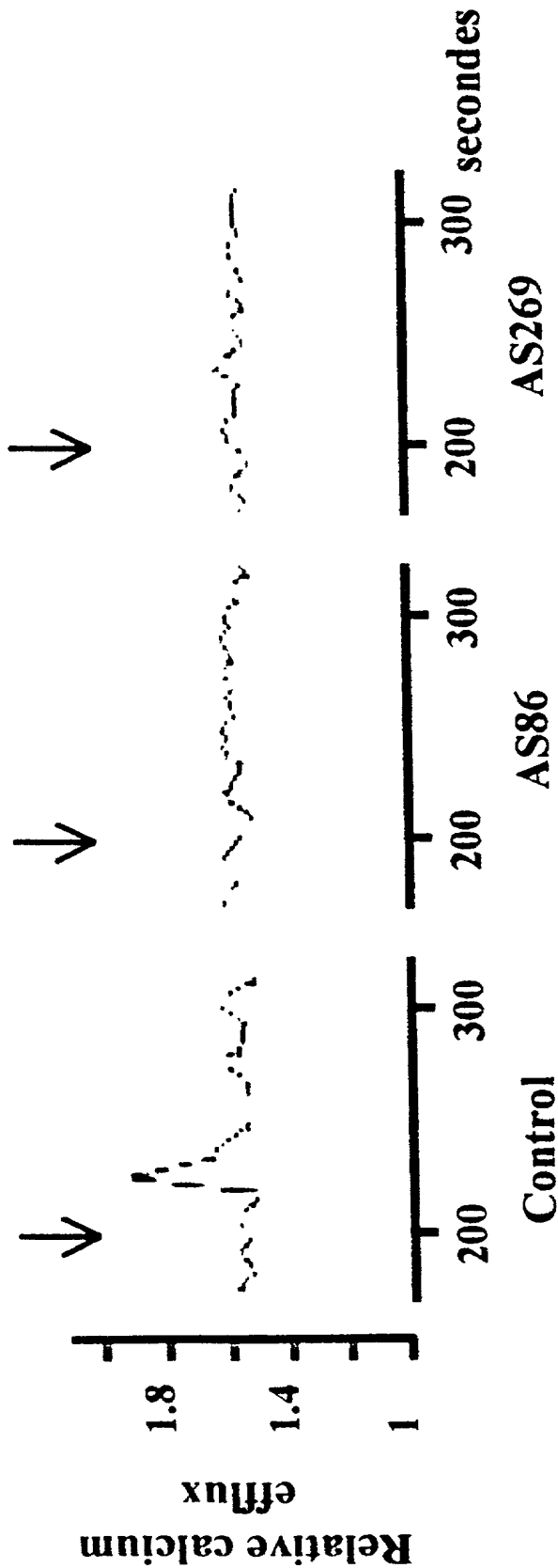


FIG - 22

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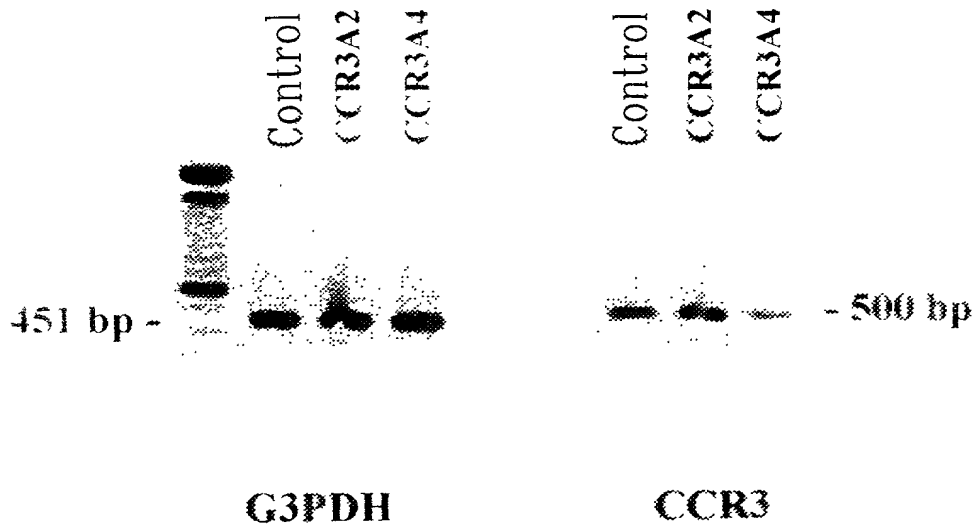
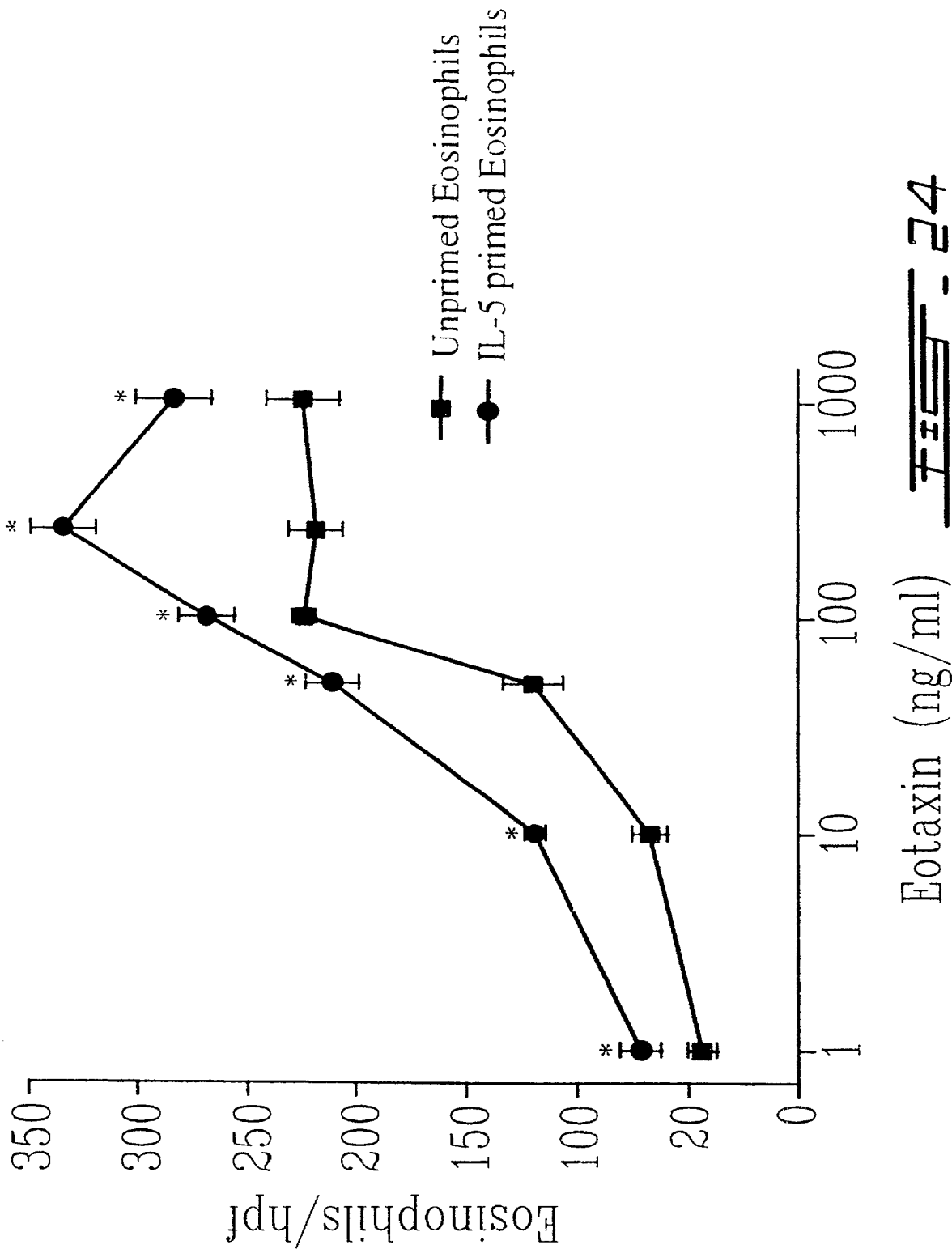


FIG. 23

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Eotaxin (ng/ml) 24

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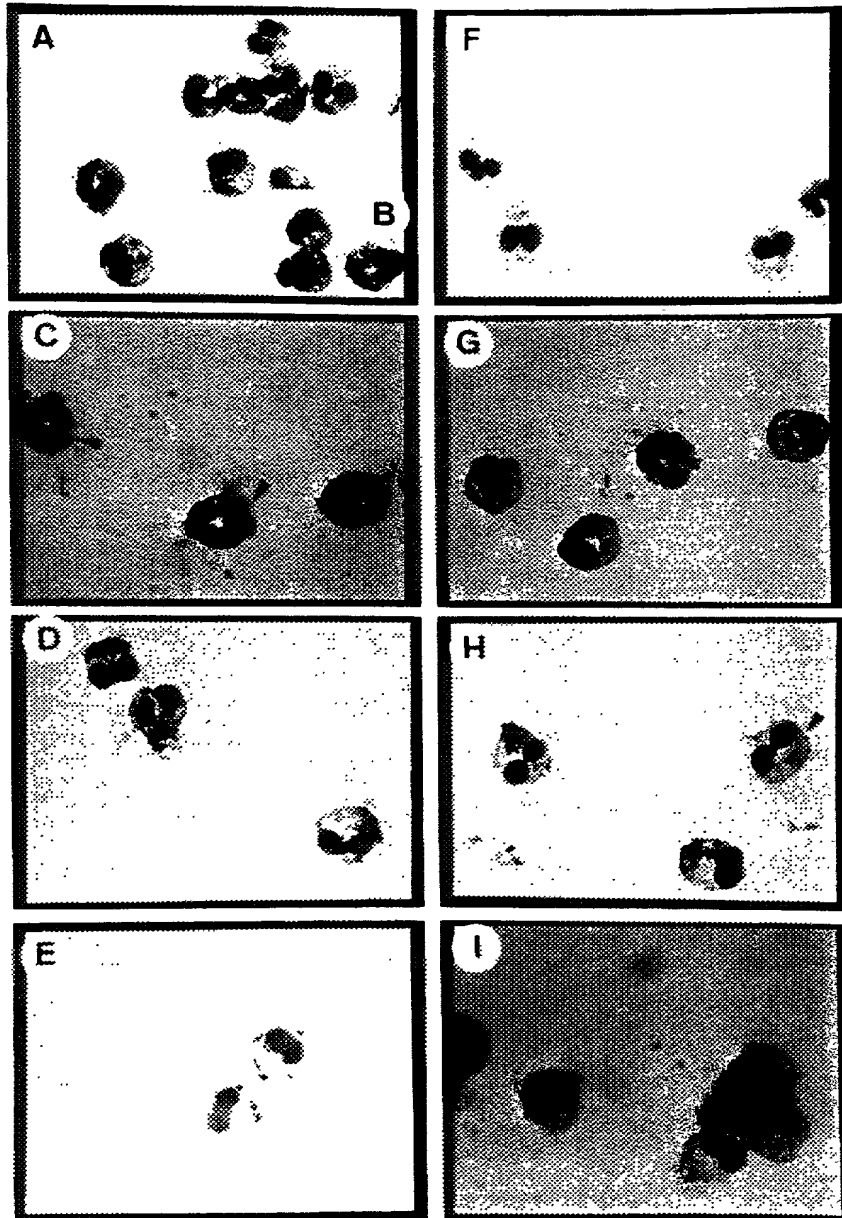


FIG. 25

Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe that I am the original, first and sole inventor (if only one name listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: ANTISENSE OLIGONUCLEOTIDES FOR TREATING OR PREVENTING ATOPIC DISEASES AND NEOPLASTIC CELL PROLIFERATION, which is described and claimed in:

[] is attached hereto.

[X] was filed on December 15, 2000 as Application No. 09/719,737
and (if applicable) was amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having filing date before that of the application on which priority is claimed;

Prior Foreign/PCT Application(s) and Any Priority Claims under 35 U.S.C. § 119

| Number | Day/Month/Year Filed | Country | Priority Claimed |
|-----------|----------------------|---------|---|
| 2,235,420 | 17 June 1998 | Canada | <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No |

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States Application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. or PCT International Applications designating the U.S.-Benefit under 35 U.S.C. § 120

| Application Serial No. | Day/Month/Year Filed | Status (Patented, Pending, Abandoned) |
|------------------------|----------------------|---------------------------------------|
|------------------------|----------------------|---------------------------------------|

Claim for Benefit of Prior U.S. Provisional Application(s) under 35 U.S.C. § 119(e)

| Application Serial No. | Day/Month/Year Filed | Applicant |
|------------------------|----------------------|-----------|
|------------------------|----------------------|-----------|

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.
ROBERT MITCHELL, Registration No. 25,007, GUY HOULE, Registration No. 24,971, PAUL MARCOUX, Registration No. 24,990, KEVIN P. MURPHY, Registration No. 26,674; ROBERT CARRIER, Registration No. 30,726; MICHEL J. SOFIA, Registration No. 37,017; FRANCE CÔTÉ, Registration No. 37,037; JAMES ANGLEHART, Reg. No. 38,796; FRANÇOIS NADEAU, Registration No. 37,570; MAX R. WOOD, Registration No. 40,388; and DAVID S. RESNICK, Registration No. 34,235; and address all correspondence to:

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Montreal, Quebec, Canada H3A 2Y3

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I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

| | | |
|---|--|--------------------------------|
| Full name of first inventor Paolo Renzi | Inventor's signature <i>Paolo Renzi</i> | Date 25/1/11 |
| Residence and Post Office address 38 Edgehill Road, Westmount, Québec, Canada H3Y 1E9 | | Citizenship Canadian |

PCT09

RAW SEQUENCE LISTING

DATE: 08/01/2001

PATENT APPLICATION: US/09/719,737

TIME: 18:44:12

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Output Set: N:\CRF3\08012001\I719737.raw

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7 <120> TITLE OF INVENTION: ANTISENSE OLIGONUCLEOTIDES FOR TREATING

8 OR PREVENTING ATOPIC DISEASES AND NEOPLASTIC CELL

9 PROLIFERATION

11 <130> FILE REFERENCE: 13424-1PCT

C--> 13 <140> CURRENT APPLICATION NUMBER: US/09/719,737

C--> 13 <141> CURRENT FILING DATE: 2001-01-29

13 <150> PRIOR APPLICATION NUMBER: CA2235420

14 <151> PRIOR FILING DATE: 1998-06-17

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35 <213> ORGANISM: Artificial Sequence

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RAW SEQUENCE LISTING

DATE: 08/01/2001

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RAW SEQUENCE LISTING

DATE: 08/01/2001

PATENT APPLICATION: US/09/719,737

TIME: 18:44:12

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RAW SEQUENCE LISTING

DATE: 08/01/2001

PATENT APPLICATION: US/09/719,737

TIME: 18:44:12

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RAW SEQUENCE LISTING

DATE: 08/01/2001

PATENT APPLICATION: US/09/719,737

TIME: 18:44:12

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Output Set: N:\CRF3\08012001\I719737.raw

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VERIFICATION SUMMARY

DATE: 08/01/2001

PATENT APPLICATION: US/09/719,737

TIME: 18:44:13

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L:13 M:270 C: Current Application Number differs, Replaced Current Application No

L:13 M:271 C: Current Filing Date differs, Replaced Current Filing Date

09719737-062904

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